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PHYSIOLOGY

VARIABILITY OF THE CROSSED EXTENSOR REFLEX (PHILIPPSON REFLEX)

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Tonic contraction of limb muscles depends on stimulation not only of their "own" proprioceptors but also of receptors of the musculature of the contralateral limb [4]. It was shown in a previous work that in decerebrate animals the crossed reflex elicited by stretching the quadriceps (Philippson's reflex) was the usual feature, and also that contraction of the contralateral extensors could occur in conjunction with its own extensor reflex and with autogenic inhibition of the muscle being stretched [3]. A question that arises is whether stretching of the quadriceps invariably exerts an excitatory effect on the nervous centers of the contralateral limb extensors. There are data indicating that the tonus of the contralateral extensors under such circumstances can not only remain unenhanced, but even be depressed [7, 8]. In the present experiments a number of cases of "inversion" of the crossed extensor reflex has been observed. Presentation of data concerning the variability of this reflex forms the substance of this communication.

METHODS

Experiments were performed on 67 decerebrate cats. The animals were placed back down on the stand, the thigh bones were fixed by means of screws in the vertical postion. Tonically contracted quadriceps muscles maintained the shins in a position of some extension depending on the extent of decerebrate rigidity. If the angle between the femur and the shin was about 90° decerebrate rigidity was considered moderate. Stretching of one of the heads of the quadriceps was achieved by passive flexion of the limb at the knee joint. The shins were connected with isotonic myographs which under these conditions recorded changes in the length of the quadriceps muscles. On all the kymograms extension at the knee joint corresponds to a downward deflection. In a number of experiments a simultaneous record of the quadriceps electromyogram was made with the help of a two-channel cathode oscillograph. Needle electrodes with an inter-electrode distance of approximately 2 cm were used.

RESULTS

The crossed extensor reflex was obtained in individual decerebrate cats with unequal ease and was of different magnitude. In some cases this reflex became apparent with slight flexion of the limb (by 5-20° in the angle of the femur and shin), while in other cases it could only be elicited on full flexion or several repeat flexions of the limb. In the majority of cases the distinctive features in the Philippson reflex were associated with the extent of the decerebrate rigidity of the hind limb extensors. The reflex could be most easily elicited and had the largest magnitude in animals exhibiting moderate decerebrate rigidity (Fig. 1, b). The reflex was, as a rule, diminished (Fig. 1, a) in animals with slight rigidity. The reflex could not be elicited in preparations with very weak torus of the extensors.

In a number of preparations (7 of 67) with weak and unstable decerebrate rigidity not only diminution but also inversion of the crossed extensor reflex was observed. When the limb was lightly passively flexed

(100-200 g) the tonus of the contralateral quadriceps was diminished as the result of which the shin was lowered somewhat. There was simultaneous weakening of the electric potentials of this muscle. If flexion of the limb was stopped, the contralateral shin returned to the original position. On increasing the passive flexion, however, inhibition of the crossed extensor tonus was replaced by its increase and the Philippson reflex was obtained. The

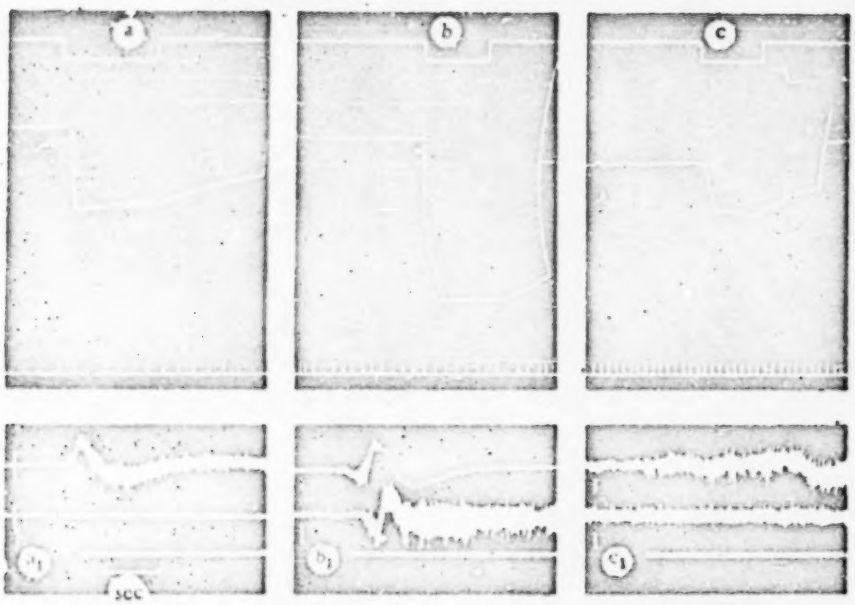


Fig. 1. Relation of the magnitude of the Philippson reflex and the extent of decerebrate rigidity. a) weak rigidity (angle between femur and shin 70°); b) moderate rigidity (90°); c) marked rigidity (125°).
Records from above down: a), b), c) stimulus marker, movements of right shin, time marker (1 second); a_1), b_1), c_1). electromyograms of the left and right quadriceps muscles, stimulus marker (passive flexion of the left limb). Calibration in all EMG records – 50 μ V.

reaction thus consisted of two successive phases. A typical example of such a response is given in Fig. 2, a.

In some experiments in which the two-phase form of the crossed extensor reflex was observed even the relatively weak stretching of the quadriceps muscle by the weight of the shin supported by it exerted an inhibitory effect on the centers for the contralateral extensors (2 cases). Termination of this stretching by slight passive extension of the limb led not to the usual gradual relaxation of tonus [3] but to a rapid contraction of the contralateral quadriceps muscle. In the case presented in Fig. 2, b, it was sufficient to raise the left shin slightly for the right limb to become extended (angle around 30°) and "freeze" in the new position.

The Philippson reflex proved to be weakened in preparations with very marked decerebrate rigidity (Fig. 1, c). It might have been supposed that a decrease in the amplitude of the reflex in these cases was connected with considerable degree of extension of the limb as a consequence of decerebration. However, not only the mechanical but also the electric reaction of the contralateral quadriceps muscle proved to be weak. As in cases of weak rigidity, so in the case of a background of very strong rigidity several instances of relaxation instead of contraction of the extensors of the contralateral limb were observed (5 cases). Inhibition of crossed extensors under these conditions, however, showed substantial differences from the form described above. In the first place, relaxation of the contralateral extensors occurred only on strong flexion of the limb (requiring 1-2 kg); in the second place, on increasing the flexion, relaxation of the contralateral quadriceps muscle was never seen to be replaced by its contraction. Inhibition of tonus in this case was also accompanied by depression of electric activity (Fig. 3).

Inhibitory forms of the crossed extensor reflex were detected particularly frequently on prolonged

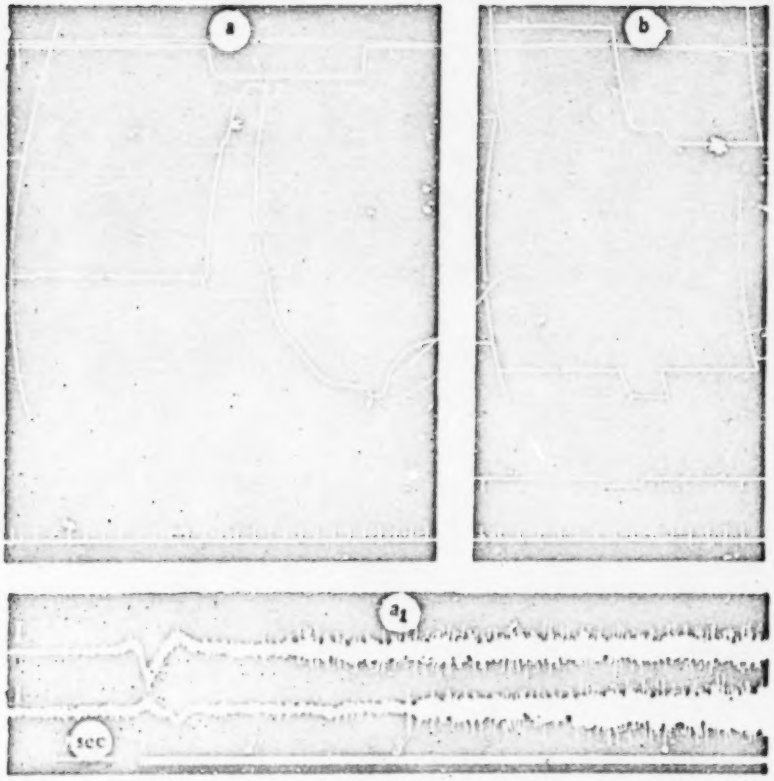
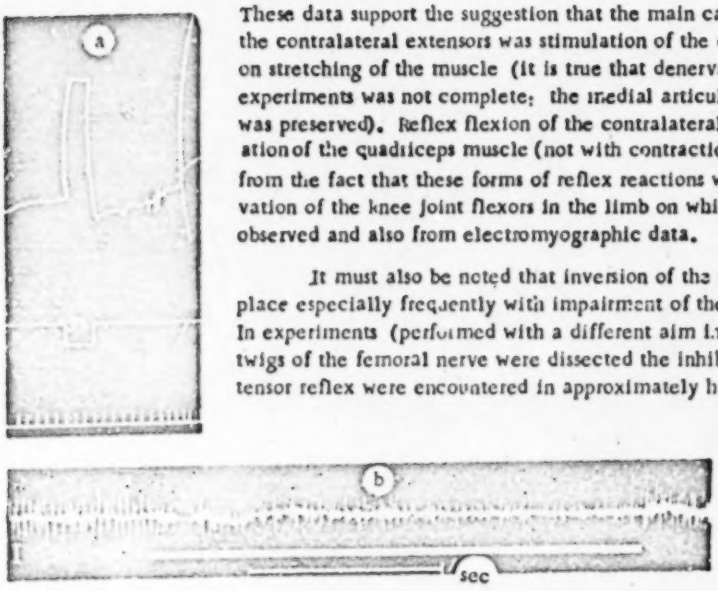


Fig. 2. Two-phase form of crossed extensor reflex against the background of weak decerebrate rigidity.
Records: a), a_1) – as in Fig. 1. Stimulus – gradual flexion of the left limb; figures indicate corresponding moments; records from above down: b) movements of the right shin, movements of the left shin, stimulus marker (gentle extension of the left limb), time marker (2 seconds). Knee flexors on the left are denervated.

observation, lasting several hours. It was then possible to establish how, in the same preparation, the character of the reflex reaction by contralateral extensors underwent changes with alterations in the extent of decerebrate rigidity. For example, at the beginning of an experiment decerebrate rigidity was moderate and flexion of the limb at the knee elicited the Philippson reflex. Later the tonus of the extensors gradually diminished and after approximately one hour the same stimulation elicited the two-phase form of the reflex. After another 1½ hours rigidity of the preparation became very weak with the appearance of continuous rhythmic movements of the hind limbs which interfered with observations. Intracranial tampons were changed and the experiment interrupted for one hour. During this time the decerebrate rigidity became very strong (angle between femur and shin 140°). At this stage, instead of the former reaction, strong flexion of the limb elicited considerable diminution in the tonus of the contralateral extensors with no signs of its increase.

Comparison of the effects of flexion of opposite limbs revealed the possibility of asymmetry in the resulting crossed extensor reflexes. If, for example, flexion of the left limb resulted in relaxation of the contralateral extensors, flexion of the right limb could result in Philippson's reflex.

The method used for the stretching of the quadriceps muscle did not exclude the possibility of "collateral" (skin, joint) stimulation. Control observations were therefore carried out in which a number of nerve trunks were sectioned (sciatic nerve above the separation of the deep thigh branches, n. saphenus, obturator nerve) and novocaine anesthesia applied to those areas of the skin whose receptors could have been stimulated on passive flexion of the limb. Both inhibitory forms of the crossed extensor reflex were observed under these conditions also.



These data support the suggestion that the main cause of inhibition of the tonus of the contralateral extensors was stimulation of the quadriceps muscle proprioceptors on stretching of the muscle (it is true that denervation of the knee joint in these experiments was not complete; the medial articular twig from the femoral nerve was preserved). Reflex flexion of the contralateral limb was associated with relaxation of the quadriceps muscle (not with contraction of the flexors). This follows from the fact that these forms of reflex reactions were fully apparent after denervation of the knee joint flexors in the limb on which the crossed reflex was being observed and also from electromyographic data.

It must also be noted that inversion of the crossed extensor reflex takes place especially frequently with impairment of the "effector" quadriceps muscle. In experiments (performed with a different aim in view) in which the muscle twigs of the femoral nerve were dissected the inhibitory forms of the crossed extensor reflex were encountered in approximately half the cases (3 of 6 experiments).

Fig. 3. Inhibitory form of crossed extensor reflex with strong decerebrate rigidity. Records from above down: a) movements of right shin, stimulus marker (passive flexion of left limb), time marker (1 second); b) simultaneously recorded EMG of the right quadriceps muscle.

Adequate stimulation of the quadriceps muscle proprioceptors can thus produce not only an excitatory but also an inhibitory effect on the centers of crossed extensors. The crossed extensors reflex posseses a variability similar to that which is well-known in the case of antagonist-muscle contractions in the course of exteroceptive reflexes [2, 6, 1]. The data obtained are difficult to interpret from the point of view of constant values of afferent impulses and nerve fibers conducting them. Although in the majority of preparations any supra-threshold stretching of the quadriceps muscle elicited contraction of the contralateral extensors, in other preparations the same stimulation elicited opposite reactions. Evidently, the basic factors which determine the variability of the crossed extensor reflex are changes in the functional state of the nerve centers. This is suggested by the dependence of the course of this reflex on the intensiveness of decerebrate rigidity and the degree of muscle stretching, the occurence of different forms of the reflex in the same preparation, and the significance of the state of the effector part of the reflex arc (observations on preparations with damaged muscle).

In decerebrate preparations the distinctive features shown by the course of spino-cerebral reflexes depend on the activity of the reflex apparatus in the preserved parts of the brain. It may be supposed that variability of simple unconditioned motor reflexes represents a phenomenon which constantly accompanies coordination of complex movements under conditions of normal reflex activity, with "reflexes and reflex apparatus of the lower levels being used for the achievement of reflex actions of higher levels" (Ukhtomsky, Vol. 5, p. 230).

SUMMARY

Stretching of one of the quadriceps was caused by flexion of one of the limbs of decerebrate cats. Variations of the length and the electromyogram of the quadriceps were recorded. The amplitude of the Philippson reflex was most at medial decerebration rigidity. The Philippson reflex was lowered both in cases of very strong and weak rigidity; in some experiments it was even inhibited. In case of flaccid rigidity the tonus of the contralateral extensors was lowered by gentle flexion of the limb (100-200 g) and then enhanced by forced flexion. In cases of strong rigidity inhibition of crossed extensors was caused only by strong flexion (1-2 kg). The crossed extensor reflex is variable just as the reflex of antagonistic muscles during exteroceptive stimulation.

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*In Russian.

EFFERENT PATHS OF THE REFLEX GASTRIC INFLUENCES ON DIURESIS

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The mechanism of interoceptive influences from the stomach on diuresis has not yet received adequate study. The present communication aims at throwing some light on the results of series experiments on 5 dogs concemed with investigation of the efferent paths of the reflex gastric influence on diuresis.

METHODS

Three dogs (Pushok, Chemy, Khmury) were subjected to denervation of the right kidney, while one dog (Elma) was hypophysectomized. In all four animals the distal ends of the ureters were externalized in abdominal skin; all dogs had gastric fistulas. In the case of a fifth dog (Jack) also with a gastric fistula, the right kidney was transplanted into the cervical region and the distal end of its ureter externalized in the chest skin, while that of the left ureter was externalized in abdominal skin.

Gastric mechanoreceptors were stimulated by means of a thin-walled rubber balloon which was introduced into the stomach through the fistula and filled with varying amounts of water – 200-800 ml and over, warmed to 38°. The urine was collected separately from the two kidneys every 15 minutes.

In part of the experiments glomerular filtration and tubular reabsorption were determined by the endogenous creatinine method. In discussing the results those experiments in which the control value for blood creatinine clearance was not less than 2.3 ml per 1 kg body weight of the dog were taken into account. Creatinine clearance is then close to inulin clearance (M. Ladd, L. Liddle and A. Cagnon, 1956 [5]). In a number of cases the results of those experiments in which the control value of clearance was 2 to 2.3 ml per 1 kg body weight of dog were also considered reliable.

RESULTS

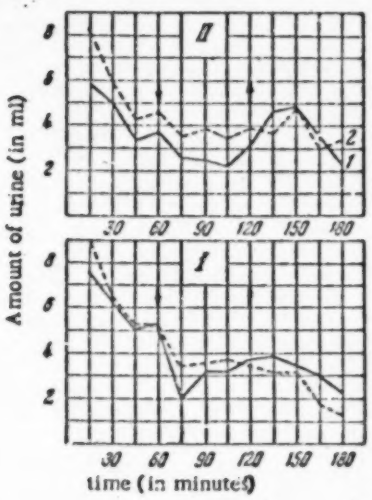
Distention of the stomach by means of the balloon produced in the dogs Pushok, Chemy and Khmury inhibition of urinary excretion both from the intact left and the denervated right kidneys. In the first few days following denervation less urinary inhibition was observed in the case of the right, denervated, kidney in some experiments as compared with the intact left kidney. In subsequent experiments (after 10-14 days) all degrees of gastric distention, as a rule, elicited uniform inhibition of diuresis both of the intact and denervated kidney.

In view of the fact that the generally practiced technique of renal denervation could leave undamaged some nerve twigs and fibers running under vascular endothelium and along the ureter to the kidney it was decided to check the results of these experiments on the dog Jack, whose right kidney had been transplanted into the neck and the left one was intact.

The figure shows the results of two experiments on this dog. Distention of the stomach by introducing 800 ml water into the baloon caused definite inhibition of diuresis of the left, intact, kidney and had little effect on diuresis of the right, transplanted, kidney (I). On stronger stimulation of gastric mechanoreceptors (balloon

filled with 1000 ml water) diuresis of both kidneys was inhibited equally (II). Results given by other experiments on this dog were analogous. Distention of the stomach by increasing the volume of the balloon to 600 or 800 ml produced definite inhibition of diuresis of the intact left kidney and had little effect on diuresis of the intact left kidney and had little effect on diuresis of the transplanted right kidney. Marked distention of the stomach (1000 ml) gave rise to inhibition of diuresis of both kidneys.

Data of experiments on the dog Cherny are presented in the table (see Table): these data show that during the distention of the stomach by means of the balloon reabsorption was growing in both kidneys. Filtration in the intact left kidney diminishes, while in the denervated right kidney it remains unchanged. Analogous results were noted in experiments on the dog Jack with the transplanted kidney (see Table). During distention of the stomach by the balloon reabsorption increases in both kidneys, filtration is diminished in the intact left kidney and increases a little in the right, transplanted, kidney.



Changes in diuresis in a dog with transplanted (1) and intact (2) kidney on stimulation of gastric mechanoreceptors.
↓ – beginning, ↑ – end of gastric distension by ballon. II and II different experiments.

Thus, urinary excretion in the intact kidney is diminished under the influence of gastric distention by virtue of increased reabsorption and decreased filtration. In the denervated or transplanted kidney diuresis can only diminish by virtue of increased reabsorption.

These data permit the assertion that with moderate stimulation of gastric mechanoreceptors the efferent arc of the reflex gastric influence on diuresis is made up of two paths: a neurohumoral one which regulates processes of reabsorption and a neural one which regulates processes of filtration. Impairment of the latter (denervation of the kidney) prevents a decrease of filtration on distension of the stomach by the balloon. These data coincide with the results of K. A. Dryagin's experiments [4] in which he obtained analogous data by studying conditioned reflex influences on diuresis, and with the inferences drawn by K. M. Bykov [3] from the experiments of his collaborators. However, with very strong interoceptive stimuli diuresis of the denervated kidney can still be altered by virtue of diminution of filtration (experiment on the dog Pushok).

Changes of Glomerular Filtration and Tubular Reabsorption on Distention of the Stomach by a Balloon (Dog)

Dog	Nature of Exp.	Time in hrs.	Filt. ml./ min.		Water Reabsorp. (% Filt.)	
			rt. denerv. kidney	left intact kidney	rt. denerv. kidney	left intact kidney
Cherny (wt. 8,1 kg)	2nd hr. exp. stomach distended by 800 ml balloon.	1-st	8.017	9.412	96.18	95.98
		2-nd	8.235	7.455	97.80	97.51
		3-rd	8.457	7.832	95.70	95.98
Jack (wt. 18,5 kg)	2nd hr. exp. stomach distended by 1000 ml balloon vol.	1 st	11.972	25.980	97.90	98.64
		2 nd	15.411	19.701	99.78	99.44
		3-rd	15.939	21.303	99.27	99.36
Pushok (wt. 6,1 kg)	2nd hr. exp. stomach distended by 800 ml balloon	1-st	15.876	13.321	99.38	98.64
		2-nd	8.197	11.502	99.01	98.82
		3-rd	15.882	24.648	98.70	98.94

For a dog of small weight (6.5 kg) distention of the stomach by a balloon 800 ml in volume was very strong stimulation. During such distention motor agitation was noted. Diminution of diuresis was considerable and occurred by virtue of diminution of filtration in both kidneys. Taking the data of this experiment as a basis it may be postulated that with strong interoceptive stimuli new neurohumoral mechanisms are brought into play which cause marked diminution of filtration.

In view of the fact that many authors ascribe to the hypophysis a leading role in the neurohumoral chain in reflex influences on the kidneys [1, 4] it was decided to investigate the reflex gastric influences on diuresis in a dog which had been subjected to hypophysectomy. Following hypophysectomy (dog Elma) inhibition of diuresis only occurred in response to extremely strong interoceptive stimulation of the stomach. A very slight inhibition of diuresis could only be produced by distending the stomach by a balloon 1200 ml in volume. It would appear that removal of the hypophysis creates serious obstacles in the path of reflex gastric influences on diuresis.

SUMMARY

It was established in experiments on dogs with denervated kidneys, as well as with transplanted kidneys, that there are two routes by which stimulation of mechanoceptors of the stomach takes place. The first is the nervous route, which regulates the processes of filtration, while the second is a neurohumoral route, regulating the processes of reabsorption. Very strong interoceptive stimulations of the stomach may result in decreased filtration in denervated kidneys. This shows the possibility of regulating the process of filtration by the neurohumoral route. Removal of the hypophysis hinders the reflex effect from the stomach on diuresis, which points to a definite role played by the hypophysis in the neurohumoral route of the efferent arch of the reflex from the stomach on diuresis.

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*In Russian.

STUDIES OF CEREBRAL CIRCULATION IN SERIES EXPERIMENTS BY MEANS OF CHANGES IN THE BLOOD PRESSURE GRADIENT IN VESSELS SUPPLYING THE BRAIN

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Regulation of cerebral circulation has, up to now, been studied mainly in short experiments and it is only recently that this problem has begun to be investigated under series conditions.

The present communication presents a brief statement of the principles underlying the method developed by us for the determination of cerebral circulation in series experiments by the gradient of the fall of blood pressure in the vessels supplying the brain.

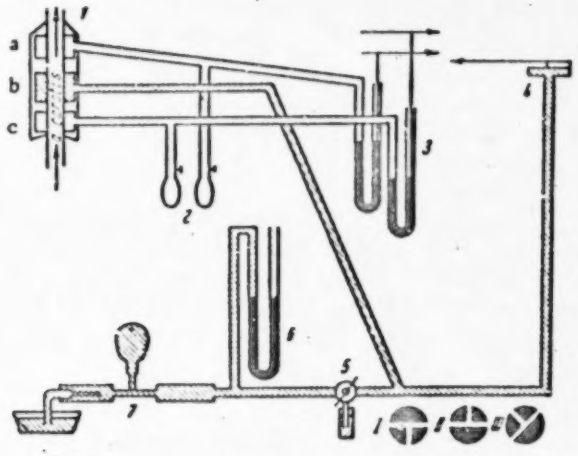

Fig. 1. Diagram of recording apparatus (explanations in text).

The premises for this method are taken from K. Hurthle who suggested investigation of cerebral circulation in short experiments using arterial pressure in the common carotid artery (M) and pressure in the vessels of the circle of Willis (M_1) as indicators. The difference in pressures ($M - M_1$) provides evidence of the rate of blood flow in the common carotid artery and the ratio of pressure in the circle of Willis to that in the aorta $\frac{M_1}{M}$ - of the state of cerebral vessels' lumen. An increase in this ratio indicates constriction, and a decrease in the ratio – dilatation of the cerebral vessels.

Our observations were carried out on dogs with the common carotid artery externalized in a skin flap

(of 35 operations 23 were successful). To avoid rupture of the carotid artery, the animals' hind limbs were bound for 10-15 days following operation.

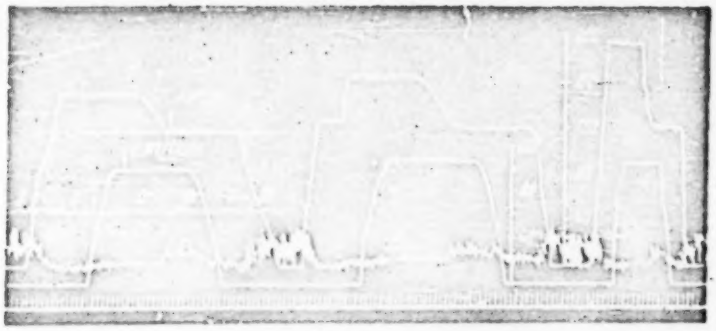

Fig. 2. Three cases of successive measurements of blood pressure in the aorta and the circle of Willis by the three-cuff method.
Records from above down: sphygmogram, pressure in "thoracic" cuff, pressure in "head" cuff, time marker (2 seconds). I), II), III), IV), V), VI) phases of sphygmogram changes. First arrow – height of pressure in the circle of Willis, second arrow – pressure in the aorta. Scale for pressure in circle of Willis 0-120 mm Hg, for aorta – 0-200 mm Hg.

Arterial blood pressure was measured by means of the apparatus shown diagramatically in Fig. 1. The recording part consisted of a folding metal capsule (1) 42 cm in length and 21 mm in diameter, in which were placed 3 cuffs: middle recording one (b), and two end ones – occluding, pneumatic ones (a, c). Each of the occluding cuffs was connected by an air system with a mercury manometer (3) and a bulb (2) for pressure production. The recording cuff (b) was connected by a hydraulic system with the recording capsule (4) or a spring manometer, and by means of a three-way tap (5) with a mercury manometer (6) and a water pump (7).

For recording, the three-way tap (5) was put in position I and part of the fluid was thus removed from the system. The capsule with the cuffs was fixed to the carotid artery; the tubes leading from it were tied to the collar. The tap was then placed in position II; pressure of about 60-70 mm of mercury was established in the system, after which the tap was placed in position III and measurements were taken. The whole preparatory procedure took 3-4 minutes.

Changes in pressure in the end cuffs are accompanied with phasic changes in the character of the sphygmogram (Fig. 2).

Phase I (background). Initial pulsation in the common carotid artery without limitation of blood flow.

Phase II. Excessive pressure created in the "thoracic" pneumatic cuff (c) led to complete occlusion of the aortal end of the common carotid artery and produced a sharp drop in the amplitude of oscillations, but their complete disappearance was never observed owing to "retum" pulsation from the vessels of the circle of Willis.

Phase III. On gradual pumping of air into the "head" pneumatic cuff (a) a moment was attained when pulsation disappeared completely; the manometer reading at that stage corresponded to pressure in the circle of Willis.

Phase IV. In the course of lowering the excessive pressure in the "thoracic" pneumatic cuff the appearance of the first pulse notch on the curve was noted and the manometer readings at that moment corresponded to maximal pressure in the aorta.

Phase V. Lowering of pressure in the "head" cuff to zero with partial occlusion of the aortic end of the common carotid artery was accompanied by a drop of the sphygmogram and diminution of its amplitude, apparently owing to friction of the blood against the vessel wall on re-establishment of outflow (in phase IV there is no movement of blood in the carotid artery since the "head" cuff completely occludes flow into the circle of Willis).

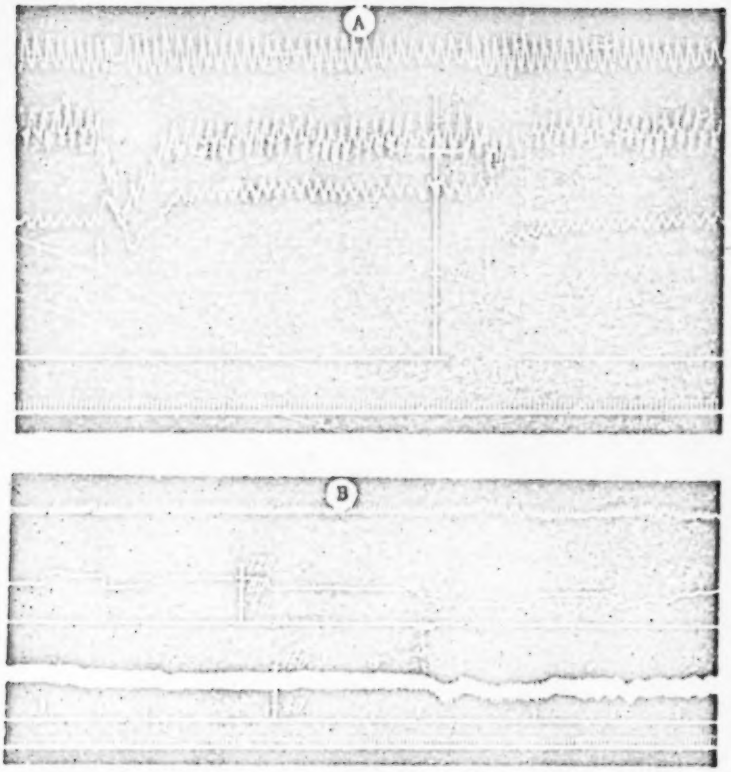

Fig. 3. Changes of blood pressure in short experiment with exclusion of branches of the common carotid artery on the side of recording.
A) Experiment on April 15, 1954; records from above down: respiration, aortal kymogram, cerebral kymogram (mercury manometer record), base line, time marker (1 second), ↑ – simultaneous occlusion of left external carotid and occipital arteries, ↓ – simultaneous removal of clamps.
B) Experiment on May 4, 1954. Records from above down: respiration, cerebral kymogram, base line, aortal kymogram, base line, time marker (1 second). Scale of pressure for circle of Willis 0-120 mm, for aorta 0-160 mm Hg. Sensitivity coefficient for the circle of Willis manometer 4.2; for aortal 6.4; first ↓ – simultaneous occlusion of the external carotid and occipital arteries on the left; second ↓ – removal of clamps; third ↑ – repeat occlusion of the same vessels; fourth ↓ – removal of clamps, fifth and seventh ↑ – clamping of the internal carotid artery on the left sixth and eighth ↓ – removal of clamps.

Phase VI (background). On lowering the pressure in the "thoracic" cuff to zero initial pulsation again appeared in the carotid artery.

In order to check the data obtained by the three-cuff method simultaneous recording of blood pressure by the direct method was carried out (15 dogs). In 11 animals the arteries were externalized into a skin flap at the time of the experiment, while in 4 dogs the vessels were externalized some time before (permanent preparation). In addition to measuring background values of blood pressure records were taken under the influence of various procedures (intravenous injection of adrenalin, infusion and withdrawal of blood). The experiments showed the similarity of results obtained by the direct and three-cuff methods. The only difference consisted of the fact that figures for pressure obtained by the indirect method were 15-20 mm Hg higher than the true

value as the result of the elasticity of the skin-vessel flap and the occluding cuff and the pressor reaction from the carotid sinus.

In order to determine on what depends the pressure measured in the cephalad end of the common carotid artery (on blood flow through the circle of Willis or on blood flow along anastomoses of the external carotid and occipital arteries) a series of short experiments on 13 dogs and series experiments on 4 dogs were carried out.

In the short experiments ligatures were placed on the internal carotid, the external carotid and occipital arteries on the side from which recordings were taken. The superior thyroid artery and small vessels branching from the region of the carotid sinus were tied; their exclusion was not reflected either on the aortic or the circle of Willis blood pressure.

After recording initial pressure simultaneous clamping of the external carotid and occipital arteries left only one pathfor blood flow to the manometer, viz. through the internal carotid artery. The general blood pressure curve (aorta) showed either no change during this procedure or, in cases where the clamp was applied somewhat carelessly, a transient fall of blood pressure (depressor reaction from the carotid sinus) was seen, followed by a return to initial levels. The blood pressure curve for the circle of Willis invariably showed a sharp rise which exceeded the initial level throughout the period of clamping (Fig. 3,A). If the clamp was removed from the external carotid and occipital arteries and placed on the internal carotid artery the curve for the circle of Willis showed a lowering of pressure. On removal of the clamp the pressure, as in the former case, rapidly returned to the initial level (Fig. 3,B).

Measurements of Blood Pressure in the Right and Left Skin Flap

No. of measurements	Left carotid (with tied vessels)				Right carotid (without tied vessels)			
	Circle of Willis	Aorta	$\frac{M_1}{M}$	$M-M_1$	Circle of Willis	Aorta	$\frac{M_1}{M}$	$M-M_1$
	In mm Hg.				In mm Hg.			
1	96	132	0.73	36	104	140	0.74	36
2	94	132	0.71	38	108	144	0.74	36
1	106	146	0.72	40	112	135	0.83	24
2	112	142	0.79	30	108	140	0.79	32
1	110	134	0.81	24	114	138	0.82	24
2	116	132	0.87	16	108	130	0.83	22

Prior to occlusion of the external carotid and occipital arteries the return blood flow from the circle of Willis thus takes place through the internal carotid artery in the direction of the common carotid artery (towards the recording manometer) as well as the external carotid and occipital arteries.

For complete exclusion of return blood flow along the supposed anastomoses through the system of external carotid and occipital arteries clamps were applied successively first to the external carotid and occipital arteries (with a rise of pressure in the circle of Willis manometer), then to the common carotid artery on the opposite side when the pressure in the aorta rose while that in the vessels of the circle of Willis dropped. The pressure returned to the initial levels immediately upon removal of clamps. On successive occlusion of the internal carotid artery on the recording side and the contralateral common carotid artery a two-fold drop in pressure was observed; the first as the result of exclusion of blood flow along the internal carotid artery, the second as the result of diminished flow of blood to the brain along the common carotid artery on the opposite side. Consequently, when connection with the external carotid and occipital arteries is maintained against the background of exclusion of the internal carotid artery the former are unable to keep arterial pressure at the initial level,

since the pressure within them is lower than in the internal carotid artery. It follows from this that the level of pressum in the peripheral segment of the common carotid artery when all the branches are kept open depends on the pressure in the internal carotid artery, and the blood flow along the external carotid and occipital arteries is directed towards the anastomoses and not in the reverse direction.

Comparison of vascular reactions against the background of occlusion of the external carotid and occipital arteries and without such occlusion was then undertaken. Test reactions were provided by the pressor reaction to occlusion of the common carotid artery and constriction of cerebral vessels on stimulation of a specially prepared sympathetic twig of the cephalad end of the vagosympathetic trunk. The pressor reaction on occlusion of the common carotid artery contralateral to recording consisted of a considerable rise of arterial pressure in the aorta and the circle of Willis; the character of this reaction remained unchanged on exclusion of the external carotid and occipital arteries.

Induction current stimulation of the cephalad end of the sympathetic twig caused a sharp rise of blood pressure in the circle of Willis while in the aorta the pressure either remained unchanged or even fell. Narrowing of the lumen on stimulation of this twig was observed both on occlusion of the external carotid and occipital arteries and without it, but quantitatively the reaction was weaker in the former case. This fact confirms literature data concerning similar reaction of cerebral and superficial head vessels to stimulation of vasoconstrictors. The isolated rise of blood pressure in the vessels of the circle of Willis shows that the lumen of cerebral vessels can alter independently of fluctuations in the level of general blood pressure.

The series experiments consisted of the following: the dogs had both their common carotid arteries externalized; on one side the external carotid artery was tied at the same time, while on the other side it remained intact. It was thus possible to compare results of measurements on the two sides. Measurement data are given in the table.

As can be seen from the table, tying of the external and occipital arteries did not affect substantially the pressure in the circle of Willis and in the aorta; therefore observation of cerebral circulation by means of the three-cuff method can be carried out without preliminary tying of these vessels.

SUMMARY

A method of graphic recording of arterial blood pressure in the aorta and the circle of Willis in series experiments on dogs is described. Measurements were taken in the common carotid artery brought out into a skin flap, to which 3 cuffs were attached: medial, recording variations in the pulse, one placed caudad and one cephalad from the medial cuff, occluding correspondingly the blood flow from the aorta and the circle of Willis. The blood pressure in the head occluding collar corresponded to the blood pressure in the circle of Willis, after disappearance of oscillations during complete occlusion of the inflow of blood from the aorta. The aortic blood pressure was determined by the appearance of the first pulse beat in decrease of the pressure in "thoracic" cuff with preserved occlusion of the "head" portion of the common carotid artery. The changes of the gradient of the fall of blood pressure from aorta to the blood vessels of the circle of Willis served as an indication of changes of the lumen of blood vessels of the brain (Hurthle's principle).

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ELECTROENCEPHALOGRAPHIC DATA CONCERNING THE CORTICAL AND SUBCORTICAL EFFECT OF MUD APPLICATIONS

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(Received December 7, 1956, Presented by Active Member AMN SSSR
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The study of the mechanism of action of medicinal muds on the organism is of great theoretical and practical interest in connection with their wide use in balneology.

The leading role played by neurogenic mechanisms in the development of the balneologic reaction of the organism to the action of medicinal mud has received general recognition.

A number of authors have investigated the central mechanisms regulating balneologic reactions [1, 2, 3, 4, 5], and the majority conclude that inhibitory processes predominate over excitatory processes in the cerebral cortex during balneologic reactions.

In order to study the dynamics of the main nervous processes during the action of mud applications on the organism, and to establish the interrelations of the cortex and subcortical centers during this action, electroencephalographic technique was used in the present work.

METHODS

Electric activity of the cerebral cortex and subcortical centers was recorded in series experiments on dogs with implanted electrodes, placed in the fronto-parietal area by the A. B. Kogan technique and in the basal region of the brain (hypothalamus) by the G.Ya.Khvoles method [7]; unipolar recording was used.

Screw electrodes with the inner shaft of silver or platinum were screwed into the frontal bone at the margin of the temporal bone and into the basal bone at the margin of the palatine bone at the base of the skull. The screw electrodes only made contact with the dura, without penetrating into the substance of the brain. Insulated leads from the electrodes were externalized in the scalp. The lead from the basal electrode was externalized through the nasal passages.

The bioelectric processes were recorded by means of a two-channel ink-writing oscillograph from the Experimental Plant of the Academy of Medical Sciences of the USSR. The medicinal mud used was the hydrogen sulfide silt from the salt-water Karasor lake (Karaganda region).

The medicinal properties of this mud have been confirmed by clinical and laboratory investigations at the Karaganda Medical Institute. The mud was applied to the shaved skin of the back or abdomen after warming it to 42°; the applications lasted for 30 minutes.

RESULTS

The experiments performed (38 experiments on 4 dogs) gave the following results.

As soon as 5 minutes after application of the mud an increase in electric activity of the fronto-parietal cortex and subcortical centers was noted. All the experimental animals showed an increase in amplitude and frequency of the waves.

Contrasting changes in the cortical and subcortical (hypothalamic) electroencephalogram occurred at the 30th minute of the mud application; there was a sharp drop in the frequency and amplitude of fast activity with the appearance of slow waves of high voltage.

The electric activity of the brain continued to diminish during one hour following the removal of the mud application; after 2 hours the initial electric activity of the cortex and subcortical centers was restored.

When mud had been applied ten-twelve times the amplitude of the cortical and hypothalamic potentials decreased and large numbers of slow waves appeared. The initial electroencephalographic picture was re-established approximately two weeks after cessation of medicinal mud applications. Changes in the electric activity of the brain under the influence of these applications were accompanied by considerable lowering of general reactivity of the animals and drowsiness.

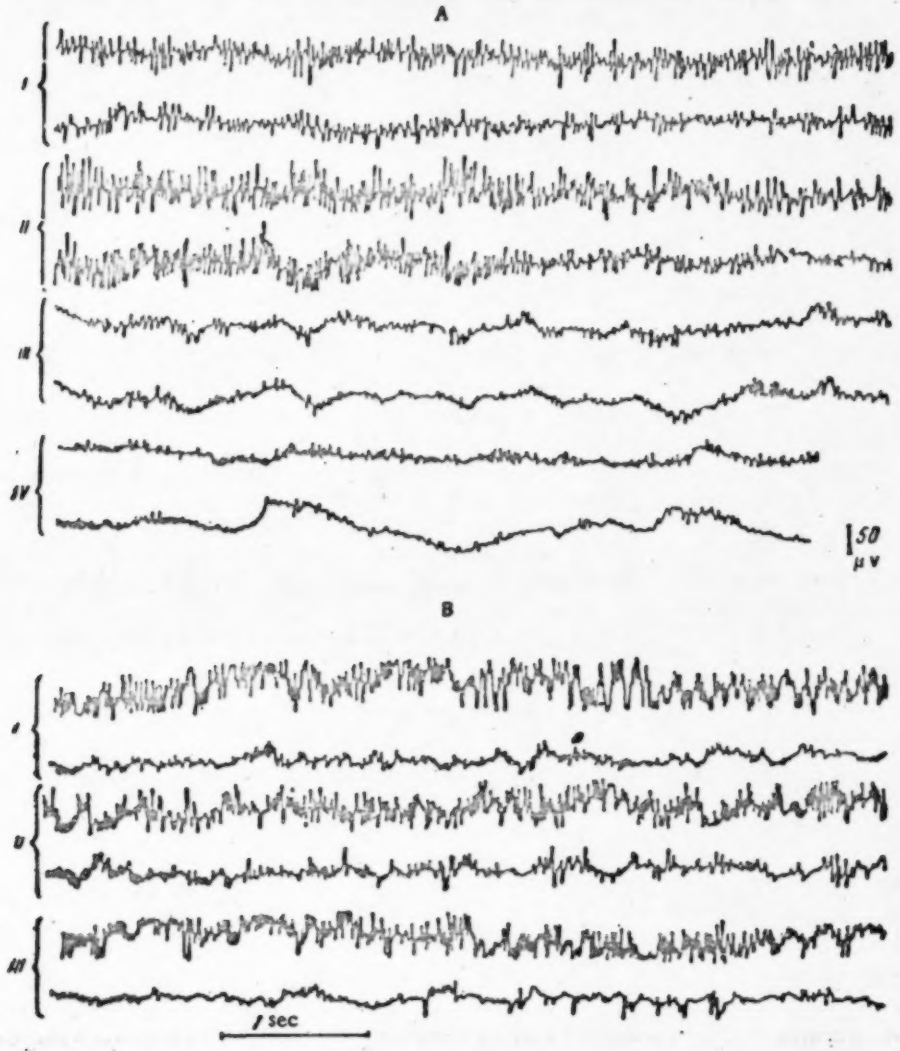

Fig. 1. A). Effect of mud applications on the electric activity of the cortex and subcortical centers. Dog Tobik. Effect on electric processes of the cortex and anterior hypothalamus. I) Initial electroencephalograms; II) 8 minutes after application of mud; III) the same after 30 minutes; IV) 60 minutes after removal of mud application. B) Effect of boiled and aired medicinal mud, warmed to 42°, on electric processes of the cortex and subcortical centers (control experiment). I) Initial electroencephalograms; II) 30 minutes after application of warmed aired and boiled down mud; III) 30 minutes after removal of temperature stimulus.

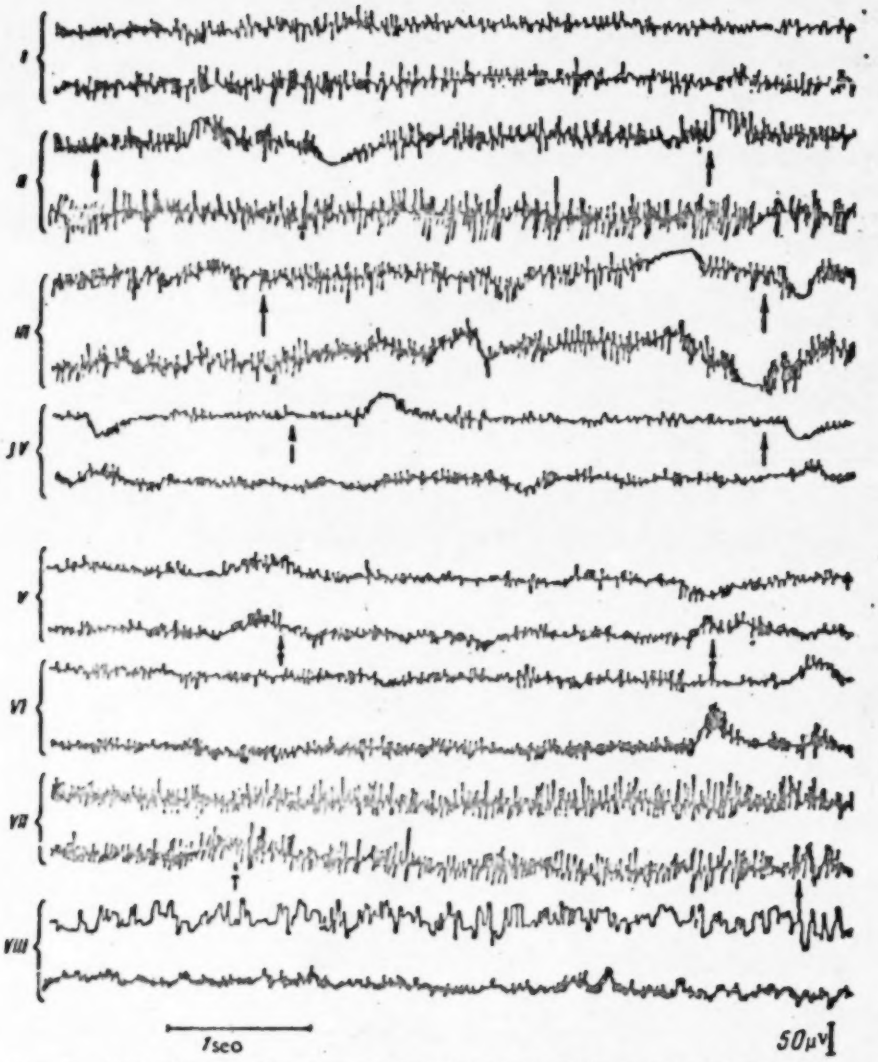

Fig. 2. Effect of mud applications on electric activity of the cortex and "anterior" hypothalamus. Dog Bulldog.

I) Initial electroencephalograms; II) with auditory stimulation; III) 6 minutes after application of mud; IV) after 30 minutes; V) 30 minutes after removal of mud application; VI) the same after 60 minutes; VII) the same after 2 hours; VIII) after 10 applications of mud over a month. ↑ – beginning, ↓ – end of auditory stimulation.

Thus, auditory stimuli of 50 cps administered prior to application of mud always evoked an increase in fast activity with increase in its voltage, both in the cortex and the hypothalamus. Moreover, single large slow waves also appeared. On cessation of auditory stimulation prolonged after-effect was seen. Under the influence of mud applications, as early as the 30th minute there was considerable lowering of the reactivity of the cortex and subcortical centers; the changes in electric activity described above were no longer elicited by auditory stimulation.

This lowering of cerebral reactivity was observed for as long as 60 minutes following removal of mud applications, and the initial reaction to auditory stimulation was only restored after 2 hours.

To illustrate the effect of mud applications on the electroencephalogram, details of experiments on the dogs Tobik and Bulldog are given below.

Dog. Tobik, weight 18 kg.

Prior to mud application (Fig. 1, A, I) the electroencephalogram showed fast activity - about 35 cycles per second, amplitude 25-100 μv - in the fronto-temporal area and activity at about 30 c/sec with an amplitude of up to 50 μv in the hypothalamus. Slower waves were practically absent from the cortex and subcortical centers. 8 minutes after application of the mud to the skin of the back the electroencephalogram (Fig. 1, A, II) showed increase of electric activity expressed in increased frequency of fast activity to 40-45 c/sec and in increase of its amplitude to 200μv in the cortex and 100-150 μv in the hypothalamus. 30 minutes after application of the mud the electric activity decreased considerably both in the cortex and in the subcortical centers; down to 20-25 c/sec and amplitude of 10-20 μv. Together with this change high voltage slow waves at 2-3 c/sec also appeared (Fig. 1, A, III). The dog was drowsy, reacting sluggishly when called. Marked lowering of electric activity still remained 60 minutes after removal of the mud application (Fig. 1, A, IV).

To exclude the effect of the temperature factor (temperature of the mud was 42°) on cerebral electric processes during applications of the mud, in a number of control experiments hot-water bottles at 42° were placed against the skin of the dog's back; in other control experiments applications were made of boiled and aired mud at the same temperature.

As can be seen from Fig. 1, B, very insignificant changes in the electric activity of the cortex and subcortical centers occurred under the influence of boiled down and aired mud, warmed to 42°.

The effect of mud applications on the electric response of the brain to auditory stimulation is illustrated by the experiment performed on the dog Bulldog, weighing 16 kg.

The electroencephalograms show that auditory stimulation (50 cps) increases the frequency and amplitude of fast activity in the cortex and hypothalamus (Fig. 2, I-II). 6 minutes after application of the mud the electric activity of the brain increased (Fig. 2, III) while auditory stimulation no longer enhanced the frequency and voltage of fast activity but only evoked large slow waves, mainly in the subcortical regions of the brain. After 30 minutes the cortical and subcortical activity diminished considerably (Fig. 2, IV) and remained at a low level—30 (Fig. 2, V) and 60 (Fig. 2, VI) minutes after removal of the mud. Normal cerebral reaction to auditory stimulation, in the form of increased amplitude and frequency of the waves, returned only after 2 hours following removal of the mud (Fig. 2, VII). Fig. 2 (VIII) shows that under the influence of mud applications repeated ten times the dog Bulldog exhibited slow rhythms - at 5-6 c/sec in the cortex and at 15-20 c/sec, instead of 35 c/sec, in the hypothalamus.

In summarizing the data of these experiments on the effect of mud applications on the electric processes of the brain we are led to the conclusion that under this influence on the organism there is predominance of inhibitory over excitatory processes in the cortex and subcortical centers. Thus, mud applications produce marked lowering of the electric activity of the brain following a brief initial enhancement of such activity; there is also considerable diminution of cortical and subcortical reactivity to auditory stimulation and development of drowsiness. Prolonged use of mud applications enhances inhibitory processes. Data obtained from control experiments with boiled down and aired mud show that the action of medicinal mud on the central nervous system can be explained only by the presence in it of active chemical components (including hydrogen sulfide), and not by the thermal effect.

The data obtained in these experiments demonstrate the participation of the central nervous system in balneologic reactions of the organism to medicinal mud applications and show the nature of the changes occurring in the CNS.

SUMMARY

The dynamics of the fundamental nervous processes of the cortical and subcortical centers were studied during mud applications to the skin surface. The method of encephalography was employed. Series experiments were conducted on 4 dogs with implanted electrodes. An initial short increase in electric activity was noted. In 5 minutes after the application of medicinal mud from Karasor lake such increase in electric activity was revealed in the cortex of fronto-parietal area, as well as in the hypothalamus in the form of increased frequency and amplitude of electric potentials. Contrary to this in 30 minutes after mud application the

frequency and amplitude of rapid oscillations was decreased, while slow waves with large amplitude appeared. The electric activity of the brain returned to the initial stage in 2 hours after removal of mud applications. These investigations prove the prevalence of inhibitory processes over excitation in the central nervous system at the time when mud is applied to the skin surface.

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PATHOLOGICAL PHYSIOLOGY AND GENERAL PATHOLOGY

CIRCULATION IN THE BLOOD OF TISSUE ANTIGENS PRODUCED BY RADIATION SICKNESS

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In our preceding publications [6, 7] we communicated the alterations in the antigenic properties of tissues resulting from irradiating animals with roentgen or gamma rays. We advanced the hypothesis that the ionizing rays alter the tissue albumins which then become antigenic stimulators and cause immunological reorganizations as, for example, a sensitization of the organism playing in this way an important role in the pathogenesis of radiation sickness.

By now we have gathered additional data describing the antigenic alterations induced in tissues by the ionizing radiation. By means of studying the reaction of active anaphylaxis with desensitization we have uncovered new antigenic capacities of the blood, the spleen, in the microsomes and hyaloplasm of the liver, in the mitochondria and microsomes of the mucosa of the small intestine of irradiated rats, as well as similar findings in the bone marrow, mitochondria and hyaloplasm of the mucous membrane of the small intestine of irradiated rabbits. *

EXPERIMENTAL METHODS AND RESULTS

The experiments were preformed on rats (150-180 g) receiving 2000 r from an experimental gamma source, the general activity of Co^{60} having 200 curies the dose strength being 34.1 r/minute. For purposes of examination, tissues were taken as a mixture from 6-8 animals. Rabbits (3-3.5 kg) were irradiated similarly with doses of 1100 r. We do not present now all the reactions of active anaphylaxis with desensitization, these illustrating the alterations in the antigenic properties of the tissues, as part of them [6, 7] has been published and others have given results analogous to those we present below.

In Table 1 are given protocols of examinations of irradiated rats 48 hours after exposure, the antigenic qualities of the tissues being studied. The framework of the experiment and the evaluation of the results follow the work done previously [7]. The table demonstrates that the blood of irradiated rats has antigens not present normally.

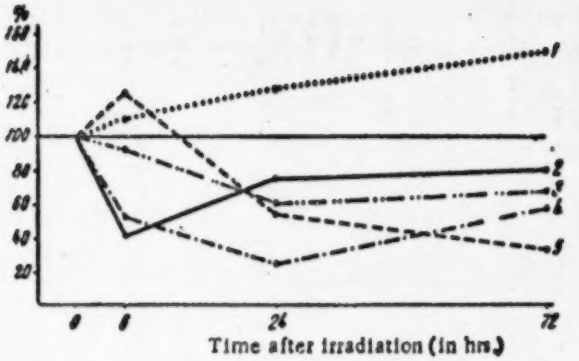
The literature [2] reveals the fact that even within the first hours after irradiation of the organism there can be observed the destruction of many tissues: bone marrow, lymphatic glands, mucous layer of the small intestine. It is also known [2] that the blood of the irradiated animals experiences an increase in its albumin content, the reason being the entrance of the albumins from the destroyed tissues. This is made all the more probable by the fact that vessel and tissue permeabilities are markedly increased in the irradiated animals [4].

To trace the transfer of tissue albumins into the blood we set up an experiment in which radioactive methionin (S^{35}) was used to label tissue albumins. The methionine solution in an amount of 0.6 μc was given subcutaneously to the white rats. After two days some of the animals were sacrificed and the radioactivity of a whole series of tissues was determined. Half the animals were subjected to an irradiating dose of 800 r.

* The cellular microstructures were separated (mitochondria and microsomes) by the method of differential centrifugation in ASL-1 and ASL-2 separators.

TABLE 1
Differences in the Antigenic Properties of Irradiated and Normal Rats

No. Guinea Pig	sensitization		Desensitization							Challenging injection		
				1st. injection		2nd injection		3d. injection				
	Antigen	dose (in cc)	Antigen	dose (in cc)	reaction	dose (in cc)	reaction	Dose (in cc)	reaction	Antigen	Dose (in cc)	reaction
1	Blood irradiated rats	0.5	Blood irradiated rats	0.1	++++					Serum irradiated rats		
2		0.5		0.025	++	0.1	±	0.1	—		0.15	++
3		0.5		0.012	+	0.1	+	0.15	—		0.15	+++
4		0.5		0.012	+	0.05	+	0.1	—		0.1	++
5		0.5		0.015	+	0.05	±	0.1	—		0.1	++
6	Blood normal rats	0.5	Blood normal rats	0.025	++++					Serum normal rats		
7		0.5		0.012	++	0.1	—				0.1	—
8		0.5		0.012	+	0.1	++	0.1	—		0.15	—
9		0.5		0.012	++	0.1	—				0.1	—
10		0.5		0.01	++	0.1	—				0.1	—
11	Not sensitized			0.05	—	0.1	—	0.1	—		0.15	—



Redistribution of Labelled Substance (S^{35}) Methionin in the Organism of White Rats after Irradiation.
1) Liver; 2) bone marrow; 3) testes; 4) mucous intestinal membrane; 5) spleen. The radioactivity of the organs of the irradiated animals at the various stated time intervals is expressed in terms of percentage in relation to the radioactivities found at similar times in similar organs of the control animals sacrificed at the same time. Each point of the curve represents the arithmetic average of four determinations. The radioactivity of the bone marrow is expressed not in terms of weights of the tissue but as a unit of its weight.

TABLE 2
Titers of Complement Binding Antibodies in the Immune Sera Against the Tissues of Irradiated and Normal White Rats

Antigens	Antisera against						
	Mucous membrane irrad. rats		Mucous membrane normal rats		Sera irrad. rats		Sera normal rats
	64	65	62	63	68	69	67
Mucous membrane irrad. rats	1/3200	1/1600	1/1600	1/3200	1/50	1/50	1/200
Mucous membrane normal rats	1/1600	1/400	1/1600	1/3200	1/5	1/10	1/50
Liver irrad. rats	1/20	1/40	1/50	1/100	1/20	1/50	1/100
Liver normal rats	1/5	1/10	1/20	1/20	1/10	1/10	1/50

TABLE 3

Results of the Complement Fixing Reaction by the Antisera Against Liver Tissue, Taken at Various Time Intervals After Irradiation (2000 r)

Sera dilutions	Antisera against: 5 hrs. after irradiation — rabbit No. 26				one day after irradiation — rabbit No. 19		Liver taken		3 days after irradiation — rabbit No. 54		rabbit No. 18			
	Antigens													
	normal liver	liver 5 hrs. after irrad.	normal liver	liver 5 hrs. after irradiation	normal liver	liver 1 day after	normal liver	liver 1 day after irradiation	normal liver	liver 3 days after irradiation	normal liver	liver 3 days after irradiation	liver 1 day after irradiation	liver 5 hrs. after irradiation
1:20	++++	++++	++	++++	++++	++++	+	++++	+	+++	++++	++++	++++	++++
1:40	++++	++++	+	+++	++++	++++	+	++	±	++++	++++	++++	++++	++++
1:80	+++	++++	±	+	++++	++++	±	+	-	+++	++++	++++	++++	++++
1:160	+	+	-	-	++	+++	r	±	-	-	++++	++++	++++	++++
1:320	-	-	-	-	-	-	-	-	-	-	+++	++++	++++	++++
1:640	-	-	-	-	-	-	-	-	-	-	+	++	++	++
1:1280	-	-	-	-	-	-	-	-	-	-	-	++	+	-

TABLE 4

Influence of Intramuscular Injections of Immune Sera (Against the Mucous Membrane of the Intestinal Membranes of Irradiated Rats Receiving 650 r) on the Course of Irradiation Sickness

Material use	No. Animals	Average blood leucocyte count (in thousands)							survived a month	avg. life of rats dying (in days)
		before irradiation	in days after irradiation							
			2	6	9	13	17	20		
Inmune serum against intestinal mucosa of irrad. rats	7	13.0	0.8	0.6	—	—	—	—	0	6.9
Same serum after adsorption	7	14.5	22.0	15.0	13.6	11.0	17.6	17.1	1	14.0
Irradiated controls	7	16.0	4.0	3.0	3.2	4.3	1.8	—	0	11.0

At the expiration of 6, 24 and 72 hours following the irradiation both the irradiated and nonirradiated animals were sacrificed and the radioactivity of their tissues was determined. At each time interval 4 rats were sacrificed. In the irradiated rats even within 6 hours time, redistribution (see Fig.) of the marked element takes place; the quantity of marked proteins in the bone marrow, mucous membrane of the small intestine and in the testes diminishes, while in the liver and spleen radioactivity rises. After a day and in 3 days the quantity of labelled substance within the liver rises, diminishing in the other organs.

The observed transfer of the labelled albumin, after irradiation, from one organ to the other and particularly to the liver can occur only by way of the blood stream. This in itself demonstrates that, when antigenic complexes arise within the irradiated animals, these abnormalities are associated with the fact that within the bed of the circulating blood stream are present tissue antigens from the intestine, bone marrow and so on. In so far as the antigenic capabilities of these tissues are altered by irradiation, these changed properties are expressed by the altered antigens now entering the blood stream.

If indeed these altered antigens from the intestine circulate in the blood and can enter the liver, then the antigenic differences between the tissues of the intestine, liver and the blood of the irradiated animals should diminish. To verify this supposition we prepared a liver antiserum and a blood antiserum in both irradiated and normal rats.

The antisera were prepared by 5-step intravenous immunization of rabbits. As antigens there were use aqueous saline extracts (1:10) from the intestinal mucosa and blood sera of rats. 8 days after the last injection of the antigen, the rabbits were exsanguinated the immune sera were obtained and with these the complement fixing reactions (CFR) were set up.

From Table 2 it is evident that the antigens obtained from the tissues of irradiated rats react in much greater titers with antisera against other tissues than do the analogous antigens of normal rats.

Thus the results of these reactions demonstrate that the antigenic differences between a series of tissues (mucous intestinal membrane, liver, sera) are less marked in the irradiated rats than the same differences between these tissues as seen normally.

The reaction alone is insufficient to form a reason for its cause. However, it is permissible to consider that the antigens from the irradiated intestine enter the blood stream and reach the liver, one of whose functions is known to be the interception from the blood of foreign albumins [1, 5]. In other words, the postradiation increase of antigenic similarity between the tissues of the liver, blood sera and mucous membrane of the intestine may be the result of the circulation within the rat organism of antigens which have been altered by the irradiation of the mucous intestinal membrane and the other tissues.

In Table 3 are presented the results of two experiments in which with the aid of the CFR test the degree of antigenic alterations caused in white rats at varying postradiation stages was determined: after 5 hours, one day and after 3 days. The last day is really the terminal stage as 2000 r caused death in the majority of animals by the 4th day. The methods of preparing the antigens, the antisera and the setting up of the CFR test have been detailed above.

From Table 3 it can be seen that antigenic differences between the normal and radiated tissues are in evidence even 5 hours after irradiation. True they are quite weak and can be caught only by observing the degree of hemolysis. Three days subsequent to irradiation the antigenic differences become quite pronounced and are revealed not only by the degree of hemolysis but also by the titer of the complement fixing antibodies.

The facts as presented here seem to be proof that irradiation causes in the organism an accumulation within the liver of circulating antigens produced from tissues, particularly the intestine, damaged by this radiation.

The data at our disposal does not permit us to state the precise role in the pathogenesis of radiation sickness played by the circulation in the blood of the abnormal antigens. However, it seems evident to us that they must act as pathological irritants and therefore inevitably must have some definite role in the pathogenesis of radiation sickness.

If this is true, then before future investigators there is uncovered a new path of influencing in some manner the pathologic process caused by the action of radiation energy. We have in mind the possibility of preparing specific immune sera against the altered antigens and, in this fashion, specifically neutralizing their pathologic action. There are enormous technical difficulties in the way of obtaining these specific antisera against the altered tissue components, as there is no certain method of adsorbing the cytotoxic antibodies against the normal components. For this reason the results of the experiment given in Table 4, can be doubly considered as being only preliminary, serving, however, to call attention to the observation that the adsorbed serum exerted a favorable influence on the course of the radiation sickness of white rats.

In the experiment we took a mixture of sera No. 64 and No. 65 against the mucosa of the intestine of irradiated rats (for titers of the sera see Table 2). One group of animals received the entire serum, the other serum adsorbed by the method of P. N. Kosyakov and others [3], the aim being to remove the antibodies against the normal tissue antigens; the third group serving for a control of the irradiation, all receiving gamma rays in 650 r dosage as described above. The antiserum was given intramuscularly the day before irradiation, on the day, and on the day following. The dose of the entire serum was 0.2, 0.5 and 0.3 cc; the doses of the adsorbed 0.5, 1.5 and 1 cc.

The results are given in Table 4.

From Table 4 it can be seen that the entire serum, containing many cytotoxins against normal tissues, had a markedly negative influence on the course of irradiation sickness in white rats. The same serum, after adsorption of the normal tissues, seemed to furnish a somewhat longer, as compared with the controls, life to the irradiated animals and appeared to prevent the development of leucopenia in them.

SUMMARY

Abnormal tissue antigens have been demonstrated as a product of irradiation of the organism. Proteins labelled with methonin S^{35} have been obtained from the bone marrow, spleen, testes and intestinal mucosa. Their passage via the blood stream to the liver has been demonstrated. Irradiation diminishes antigenic differences. As irradiation sickness progresses, these abnormal antigens must play an important role in the progress of the disease.

Adsorption of the antigens against the normal tissues opens a tentative path into very interesting future research for possible treatment of irradiation sickness.

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HISTAMINE CONTENT OF BLOOD AND LYMPH AND THE ACTIVITIES OF HISTAMINASE AND CHOLINESTERASE DURING THE DEVELOPMENT OF EXPERIMENTAL INFLAMMATION

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According to many authorities [2, 3, 6], pathological states of various divisions of the nervous system, as well as in the course of some internal diseases, are accompanied by functional disturbances of the autonomic nervous system and the amount of their mediators present in the blood becomes elevated. This rise is regarded as an accumulation of an excess of neurohumoral factors at the site of their formation as a consequence of pathological irritation and the consequent migration of these substances into the blood stream.

A great deal is known concerning the histamine content of blood and tissues and the activities of the enzymes histaminase and cholinesterase.

However, very little is known about the activities of these enzymes in the lymph, even though the composition of the lymph reflects the events going on in the tissues of both the normal organism and also when its functioning has been upset.

The aim of the present study is to investigate the blood and lymph as to their content of histamine and the activities of histaminase and cholinesterase during the course of an experimental inflammation of the small intestine.

EXPERIMENTAL METHODS

The experiments were performed on 88 cats of approximately the same age and weight. The animals had a laparotomy under aseptic conditions and turpentine in quantities of 0.1 cc was introduced into the wall of the small intestine and into the lumen in some 10-12 places.

As inflammations developed the animals were utilized in short experiments, a new animal being used each day from the group operated simultaneously.

In this fashion, we were able to trace the changes in the histamine-histaminase system and the cholinesterase activity during the inflammatory alterations taking place in the small intestine at the end of 3 hours, and then, 1, 2, 3, 5, 7 and 10 days after the time of injection of the turpentine. The cats were immobilized by injection of lycopodium into the carotid arteries. Blood for examination was drawn from the carotid artery, and lymph was drawn from the thoracic duct after a preliminary laparotomy. Histamine was determined by the biological method with a very slight modification of the method and instrument of V. M. Borovsky [4], histaminase activity – biological and chemical methods, cholinesterase activity – biological method [7].

Histamine Content, Activity of Histaminase and Cholinesterase in the Blood and Lymph of Cats after Injection of Turpentine (Average Values)

Time after introducing turpentine (in hours)	No. Animals	Histamine content						Histaminase activity (γ histaminase destroyed by histamine)						Cholinesterase activity					
		in blood			in lymph			in blood			in lymph			in blood			in lymph		
		in γ %	In % content normal animals	accretion (+) deficit (-) in %	in γ %	In % content normal animals	accretion (+) deficit (-) in %	in γ %	In % content normal animals	accretion (+) deficit (-) in %	in γ %	In % content normal animals	accretion (+) deficit (-) in %	in Shelter units	In % normal animals	accretion (+) deficit (-) in %	in Shelter units	In % content normal animals	accretion (+) deficit (-) in %
Clinical health of animals		1.31	100		0.75	100		1.19	100		1.56	100		0.39	100		0.28	100	
3	12	1.59	121	+21	0.95	127	+27	3.58	301	+201	4.12	264	+164	0.31	79	-21	0.24	86	—14
24	12	1.55	118	+18	1.10	116	+ 16	2.44	205	+ 105	3.11	199	+99	0.20	51	—49	0.16	57	—43
48	13	3.14	240	+140	1.21	127	+ 27	2.30	119	- 19	2.88	185	+65	0.18	46	—54	0.16	57	—43
72	14	3.40	260	+161	1.73	182	+ 82	0.80	67	—33%	1.73	111	+11%	0.19	49	—51%	0.14	50	—50
120	13	1.56	119	+19	0.75	100	0	2.54	213	+113	3.68	197	+97	0.36	92	—8	0.21	75	—25
168	12	1.34	102	+2	0 94	99	—1%	3.34	197	+97	3 66	225	+135	0.35	90	—10	0 27	96	—4
240	12	1.40	117	+17	0.76	80	—20	2.73	229	+129	3.40	218	+118	0.40	103	+3%	0.36	129	+29

EXPERIMENTAL RESULTS

The average results obtained were compared with the base content of histamine, histaminase activity and cholinesterase of the same individual experimental animal (see Table). As this table shows, the histamine level rises in cats after turpentine has been introduced into the small intestine, its content in blood and lymph growing gradually to a maximum after 2-3 days, beginning to diminish after 5 days but not returning to normal, and by the 10th day the histamine content of the blood was higher than in lymph.

After introducing the turpentine into the small intestine the histaminase activity in the blood and lymph changed almost parallelly; within 3 hours there was observed a marked rise (in blood – 201%, in lymph – 164%), then a gradual decrease, and within 3 days the blood showed even a 33% decrease while the lymph had an insignificant increase of 11%. In the succeding days there was again an increase (after 10 days, in blood – 129%, in lymph – 118%).

The cholinesterase activity in cats after the inception and development of inflammation decreased in both the blood and lymph in about the same proportion; being most marked after 2-3 days and least – after 7-10 days.

Histologic studies confirmed the presence of the inflammatory process in the small intestine; within 3 hours there were the signs of beginning inflammation, definite edema of the mucosa and submucosa, blood vessel engorgement; after a day marked edema of the submucosa, sloughing of the epithelium with surface villi and focal mucous necrosis; after 2 days – phlegmonous, submucous, and intramuscular sloughing with leucocytic dissolution. Later studies of the inflamed tissues showed a copious cellular infiltrate, presence of a fibrinous exudate, hemorrhages and necrotic foci in the intestinal wall.

The rise in histaminase activity within the blood and lymph within the first hours after introducing the inflammation-producing agent, in all probability, must be associated with the beginning of the active hyperemia, while later (by the 2d-3d days) the histaminase activity may be the consequence of dystrophic processes proceeding in the tissues and leading to the formation within them of histaminase, this latter being then destroyed.

The rise of the histamine level within the blood and lymph can be explained by the formation or liberation of large amounts of histamine as well as of the depression of histaminase activity.

Depression of the cholinesterase may be a consequence of the rise of the histamine, as it is well known that histamine has a strong anticholinesterase activity [1, 5].

SUMMARY

Experimental inflammation in cats raised the histamine level of blood and lymph. The histaminase activity rises simultaneously while that of cholinesterase was depressed.

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ELECTROPHYSIOLOGICAL CHARACTERISTICS OF THE ACTION OF FOREIGN BLOOD UPON SPLENIC INTEROCEPTORS

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When investigating the mechanism establishing the interoceptive reflexes, great significance must be attached to clarification of the roles played by the individual links of the central nervous system leading to the completion and fulfillment of the reflexes.

According to the data of V. N. Chernigovsky [10] and his co-workers – V. A. Lebedeva [7], G. A. Kovaleva [4], as well as V. A. Alekseev [1], in decerebrate animals, when interoceptors of the internal organs are stimulated, there are vasomotor and respiratory responses but no basic or even substantial alterations occur. It should be noted that with high decerebration, when the transection is made superior to the anterior colliculi of the corpora quadrigemina, the reflex arc coming from the interoceptors does not appear to be markedly affected.

V. N. Chernigovsky in analyzing his results came to the conclusion that the principal prerequisite for the preservation of the interoceptive reflexes is the intactness of the visual colliculi.

Of great interest are the studies of R. S. Vinitskaya [2, 3] who showed the presence of a regulating influence within the hypothalamic region, this determining the intensity of the interoceptive reflex response.

The present investigation used the method of recording bioelectric potentials within certain subcortical areas as a partial index of the reaction of the organism to interoceptive irritations produced by irrigating the spleen with foreign species blood.

EXPERIMENTAL METHODS

The experiments were performed on dogs as short experiments. Under ether anesthesia, the skull had trephine openings made in it, ebonite plugs being used to close them and electrodes being inserted through them. As electrodes silver wires were used, their diameter being 0.2 mm and their manufacture following the method of A. B. Kogan [5].

The precise place of insertion of the electrodes was determined by studying anatomic preparations of the fixed brain with the additional aid of serial frontal sections, as well as by histological studies of the areas where the insertions were actually made.

The bioelectrical activities of the subcortical regions were registered by means of a single channel oscillograph recording on photopaper moving 2.8 cm per second. The sensitivity of the instrument – 2.6 μv for every 1 mm declination of the ray.

The experiments were conducted in a screened chamber.

From the moment of inserting the electrodes into the brain tissues to the beginning of recording of the potentials in the individual experiments, from 1.5 to 2.3 hours would elapse. This greatly exceeded the time needed to free the subcortical EEG's from the currents of injury (10-15 minutes) as established by A. B. Kogan [6].

The experiments were begun only after stabilization of the background of electrical activity.

The spleen circulation was also isolated under ether anesthesia, by the method of V. N. Chernigovsky. The foreign blood used came from rabbits, the citrated blood being introduced into the perfusate from a vessel suspended on the wall of the oscilliographic chamber and allowed to flow for 5-10 minutes, thus irrigating the spleen.

The bioelectric potentials of the subcortical structures were correlated with kymographic recordings of the arterial pressure and were taken before and during perfusion as well as well as 5, 10 and 20 minutes after the spleen had been perfused with the foreign blood.

EXPERIMENTAL RESULTS

In the experiments the electrical activities of the visual colliculi (anterior, medial and ventral nuclei as well as the hillock), the subcollicular region, the caudate nucleus, the corpora quadrigemina and the pons Varoli were recorded.

It was established that perfusion of the isolated spleen by the V. N. Chernigovsky method using foreign (citrated rabbit) blood led to numerous alterations in the enumerated structures.

The changes were greatest in the electrothalamograms showing themselves in the increased amplitude of the slow waves – 3-7 cps constituting their specific characteristic (Experiments Nos. 90, 97 and 100), occasional discharges of sharp, spike-like negative waves (Expt. No. 90) slow waves with sharp peaks (Expt. Nos. 95 and 97), and voltage increases of all components of the electrothalamogram.

These changes coincided with the beginning of the perfusion, the electrothalamogram quieting by its end (Fig. 1).

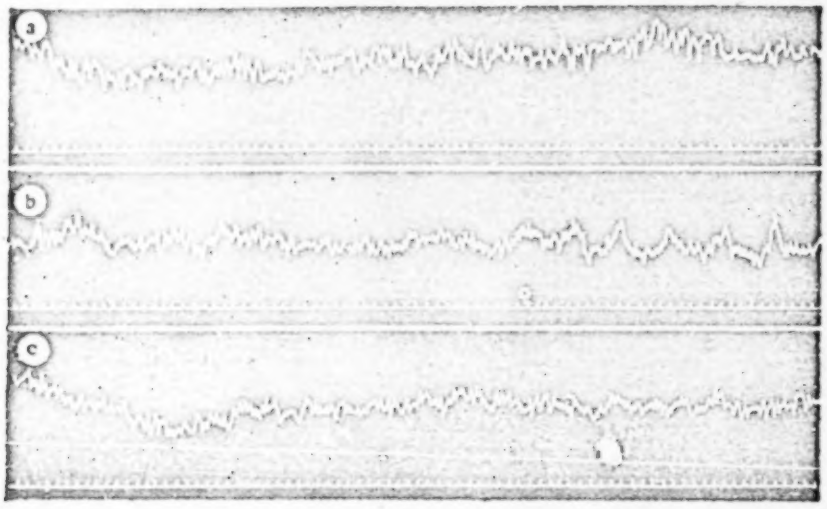

Fig. 1. Bioelectrical activity of the thalamic region in the dynamics of stimulation produced by foreign blood stimulating the spleen interoceptors (Expt. 95, October 12, 1952). Dog weighed 22 kg.
Tracing records electrical activity of the ventral nucleus of the visual colliculus; a) before perfusion of spleen with foreign blood; b) during irrigation; c) at end of perfusion. Time marker (1/20 second).

In the instances described, the introduction of the rabbit blood into the perfusing fluid led to a sharp reaction on the part of the cardiovascular system, the response having a pressor-depressor character.

In those experiments where the background activity was characterized by low amplitude bioelectric waves (Expts. Nos. 97a, 105, 109 and 110), perfusion with foreign blood did not provoke activity changes. The hemodynamic response in these cases was also feeble.

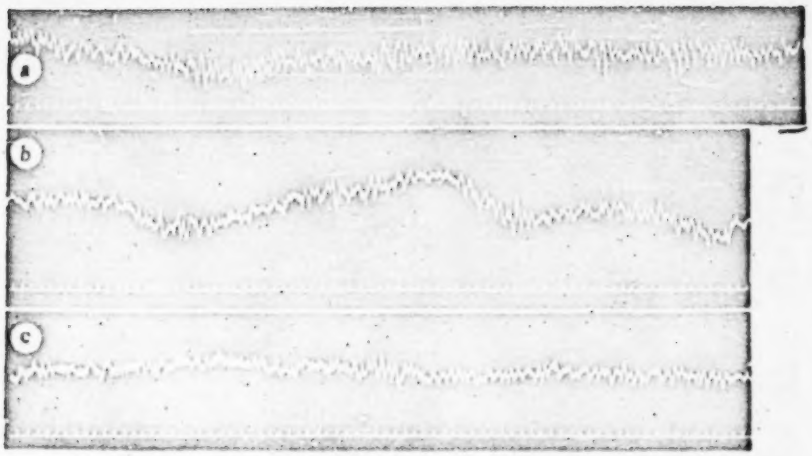

Fig. 3. Bioelectric activity of the pons Varoli in the dynamics of the effect of foreign blood perfusion upon the splenic interoceptors (Expt. No. 95, December 10, 1952). Dog weighed 22 kg.
Record of electrical activity: a) before irrigating spleen with foreign blood; b) from the beginning of the irrigation; c) at end of irrigation.

Under the conditions of our set-up, the experiments failed to show a strict parallelism in the bioelectrical alterations of some of the brain regions studied. Thus, perfusion of spleen with foreign blood increased the bioelectrical activity of the pad of the visual colliculus in some instances when the head of the caudate nucleus was completely unreactive, and vice versa.

In one of our experiments (No. 110a) the blood appeared to have no influence on the nature of the thalamogram, yet the potentials from the nucleus griseus revealed a sharp increase in amplitude (7 and 8 fold), going on into an "electric storm".

Along with this the complex method of potential recordings has succeeded in establishing the presence of a single direction of the EEG changes of the central region of the thalamus and the grey matter in the left wall of the third ventricle (marked rise in electric potentials along with a pressor arterial response) which, in all likelihood, indicates a single nerve process underlying the activity.

All this gives us the right to postulate that stimulation of splenic interoceptors with foreign blood under the conditions of the perfusion experiment leads to complex functional alterations in all the divisions of the brain investigated. This is reflected by the EEG tracings taken from the brain divisions studied.

The changes met with in association with interoceptor stimulation of the spleen by means of foreign blood perfusion clearly reveal electrical activity in the thalamic region with frequent spiking, indicating that they are not only a reflection of local changes associated with synaptic stimulation (voltage alterations of the basic components of the tracing) but also a reflection of afferent impulses going through the visual colliculi to other divisions of the brain. In this last instance we are discussing that type of cerebral activity that V. S. Rusinov [9] calls "running waves of excitation" and which manifests itself by axon-like discharges.

Putting together the electrophysiologic pictures obtained from the various subcortical levels, we allow ourselves to suppose that the afferent impulses aroused by the action of the foreign blood upon the splenic receptors direct themselves to the subcortical regions, placing the different levels at times into reciprocal, at times into synergistic correlations, thus creating a whole subcortical mosaic (stimulation of some regions-inhibition of others) which basically will determine the response of the organism to the interoceptor stimulation.

The use of stereotactic instruments combined with the principle of simultaneous recording of various potentials from the subcortical regions, analogous to the method worked out by M. N. Livanov for the cortex of the brain [8], will permit the answer to this whole question to be given in detail.

It is interesting to observe that in those instances where the EEG's showed signs of returning to base level activity after the perfusion, there was also observed a marked tendency for restoration of the arterial blood pressure (Fig. 2).

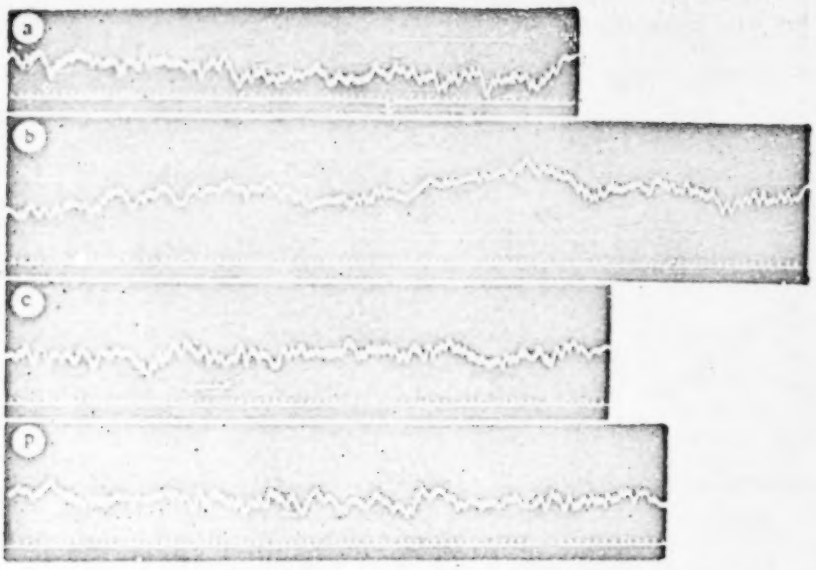

Fig. 2. Bioelectric activity of the thalamic region in the dynamics of effect of perfusion with foreign blood of the spleen interoceptors (Expt. No. 100, December 21, 1952). Dog weighed 9 kg.
Tracing recorded activity of medial hillock: a) before perfusion of spleen; b) beginning of perfusion; c) 10 minutes after the perfusion; d) 20 minutes after the perfusion.

During splenic irrigation with the heterophilic blood the EEG of the nucleus griseus also sharply changes its character.

The usual picture of low amplitude rhythm of 20 cps waves is replaced by a slow 2-6 cps rhythm, with a voltage of from 6 to 35 μv, while by the end of the perfusion, after the "electric storm" – single slow waves with a period from 0.5 to 1 second and a voltage of 70-75 μv, alternate with waves having a rhythm of 4-5 cps.

It must be noted that in these experiments where the electrodes were sunk into the nucleus griseus we observed a more marked response to the foreign blood, which confirms the conclusion of R. S. Vinitskaya [2] as to the influence exerted by the hypothalamic region upon the intensity of the interoceptive reflexes.

Of interest were the bioelectric potential alterations in the caudate nucleus (head) as the dynamics of splenic irritation with foreign blood unfolded. Here, as with the potentials of the thalamic region, there was observed a correlation between sharp responses of the EEG with the hemodynamic status.

At the same time, comparison of EEG's from the pons Varoli with kymograms of the arterial pressures during the period of activity by the foreign blood perfusion fails to establish any consistent correlation. Here there could often be seen EEG's changing in the absence of hemodynamic response to the heterophilic blood (Fig. 3).

Accepting as fact that electrophysiologic changes reflect nerve processes, we attempted to record within a single experiment the simultaneous bioelectric potentials of various regions of the brain, the effort being to analyze any possible intercorrelations between the subcortical regions being established while the splenic interoceptors were being stimulated by the foreign blood.

As we had only a single channel installation, we could not make the recordings truly simultaneous. We were forced to record the waves in sequence, at intervals of 5-10 seconds, switching the current at the distributor bar.

SUMMARY

Electrodes were placed into precisely predetermined subcortical levels of dogs during short experiments. Their spleens were then perfused with rabbit blood after this organ had been humorally isolated. The EEG tracings were taken simultaneously with arterial blood pressures.

It was noted that the most pronounced changes were caused in cases where the hemodynamic responses were also most rapid.

The theory is advanced that the afferent stimuli from the splenic interoceptors sets up a regular mosaic of reciprocal and synergistic subcortical afferent waves of stimuli, the sum total of these determining the response of the organism to the stimulus.

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CONCERNING THE MECHANISM PRODUCING AN ADRENALIN MYOCARDITIS

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Spinal shock expresses itself, as is well known, by severe reflex suppression mainly below the level of sectioning of the spinal cord. This permits the use of spinal section as a method for the temporary exclusion of nerve centers in the distal portion.

Our previous studies [1] have shown that in rats whose spinal cord had been severed at the $C_8 - T_1$ level and who had thus had induced in them spinal shock, injections of adrenalin during this period of shock will either not induce the development of myocarditis at all or, at most, induce it in only a light form. This permits the supposition that the inflammatory myocardial process is suppressed in these experiments because the spinal centers of cardiac innervation (T_1–T_5) are in the shock zone and, therefore, the reflexes associated with them are greatly hampered. Such a supposition is supported by the results of experiments with sectioning of the spinal cord at the lumbar level and with experiments in which decortication is performed: severing the lumbar cord does not prevent adrenalin myocarditis; decortication, which intensifies reflex activity, also makes the adrenalin myocarditis develop most severely. It still did not clarify the cardiac alterations accompanying these three indicated operations and did not reveal the relations between these changes and the development of adrenalin myocarditis.

The present study was undertaken in an effort to answer these questions, and we have repeated our previous studies, with some additional work and use of the ECG machine.

EXPERIMENTAL METHODS

The spinal cord was severed at the $C_8 - T_1$ level in 27 rats. ECG's (the three standard and fourth chest leads) were taken before the operation and 24 and 48 hours after the operation. Then, the "myocarditis" dose of adrenalin (0.5 cc) was given intramuscularly and the ECG was repeated (40 minutes and 24 hours after the injection).

EXPERIMENTAL RESULTS

Of the 27 rats operated, 2 died within two days of the operation, 11 perished shortly after the adrenalin injection, and the remaining 14 were sacrificed 24 hours after the injection of adrenalin. The rat hearts were studied microscopically (frozen sections stained with Sudan III and hematoxylin). In 7 rats no signs of myocarditis could be found, in 6 rats the myocarditis appeared to be quite mild, and only in one rat was it marked. In the control material, the same dose of adrenalin given to 52 healthy rats induced myocarditis in 46 rats.

In our previous experiments (21 operated rats) myocarditis did not develop in 12 rats, and was only

mildly evident in 9. In the control experiments myocarditis developed as a rule (39 out of 43 rats receiving 0.5 cc adrenalin and 151 out of 153 receiving 0.8 cc).

This demonstrates that in two groups of experiments with sectioning of the spinal cord at level $C_8 - T_1$ the response with a development of a myocarditis to an injection of adrenalin was almost eliminated.

ECG's taken 24 and 48 hours after the operation (before injecting the adrenalin) showed considerable abnormalities. All rats had bradycardia of varying degree, and some had arrhythmias.

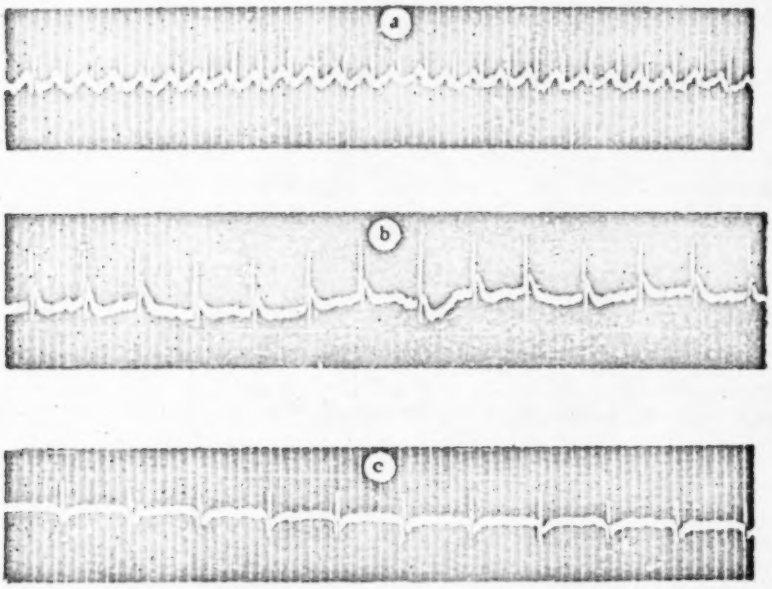
The normal rat heart rate is 480-500 beats per minute. In rats the spinal cord of which had been severed at the C_8–T_1 level the pulse varied from 160 to 400 beats per minute.

The ECG changes indicated severe metabolic disturbances and conduction myocardial disturbances (lowering or elevation of R wave, S–T interval shifts in the chest and, sometimes, in the standard leads, fragmentation of the QRS complex in the chest lead, biphasic or inverted T).

It should be noted that after the indicated operation the ECG waves in the rats altered much as after injections of large doses of adrenalin.

On the ECG taken 40 minutes after the adrenalin injection, there could be noted an acceleration of the pulse by 20-60 beats per minute while in the others there could be observed an aggravation of those changes noted after the operation. Twenty four hours after the injection the pulse either returned to normal post-operative levels or else the bradycardia became more pronounced. In rats with a developing myocarditis the ECG would reveal appearance or deeper inversions of the T wave. Rats not developing a myocarditis showed no further ECG alterations (Figure).

The appearance of the bradycardia after the spinal cord sectioning above the level of cardiac innervation is evidence in our experiments of the depressed state of the sympathetic centers in this zone.



Experiment with introduction of adrenalin after operation severing the spinal cord at level $C_8 - T_1$, rat No. 1062 (ECG taken in chest lead IV).
a) ECG before operation. Pulse is 480 per minute. R is 0.5 mv; b) ECG 24 hours after the operation. Pulse is 260 per minute. S-T interval shift. R is 1.1 mv. T is inverted; c) ECG 24 hours after injecting 0.5 cc adrenalin. Pulse is 170 per minute. T is isoelectric. No myocarditis was seen histologically.

It is well known that injuries precisely in the cervical spinal cord region can give a bradycardia down to 40 systoles per minute [3, 4].

The variations in the bradycardia observed by us may be due to variations in the depth of shock as well as due to variations in the locations of the cardiac sympathetic centers.

In order to observe further cardiac state and activity after section of the spinal cord at level of C_8-T_1, 30 rats were kept as long as possible after the surgery. The rats were catheterized every 6-8 hours, a hematuria being observed early. In most of the rats (26) the bradycardia became more marked and the ECG changes observed early became more marked and could be seen in all four leads. The rats died within 1 to 9 days after surgery.

Histologic examination of the rat hearts shows that even within the first day after operation the myocardium reveals a marked hyperemia (neuroparalytic ?) and marked cloudy swelling of the muscular fibers. In cases of death in later stages the myocardium showed extravasations and increasing albuminous dystrophy.

It must be assumed that the muscular changes seen in the myocardium are only a partial expression of the profound metabolic alterations which are reflected in the ECG within the first day of the operation. It is most unlikely that the adrenalin-sensitive biochemical systems within the muscle cells and the myocardial receptors remain intact functionally with such myocardial alterations. For this reason it is difficult to determine where the reflex arc was severed and so enabled the adrenalin not to have the usual myocarditis effect. This is especially difficult in the short experiments where the cessation of the existence of central impulses cannot be differentiated from the spinal section itself affecting the spinal cardiac centers.

In our previous experiments [2] we established that introduction of sympatholin (adrenalin antagonist) as late as 5-8 minutes before the adrenalin injection prevents the possibility of an adrenalin myocarditis developing. It should be noted that intramuscular injection of the sympatholin is accompanied by bradycardia. In some of the rats this bradycardia could be observed within 5 minutes of the injection (2 rats), while within 1 hour the bradycardia was present in all 9 rats.

It is possible that a part of the antagonistic action on the heart by the sympatholi n is due to inhibition of the sympathetic centers of cardiac innervation.

The data presented above and the conclusions drawn from it seem more convincing when juxtaposed with the results seen after the spinal cord had been transected at the lumbar level. When this is done, the spinal cardiac sympathetic centers remain outside the zone of spinal shock. The spinal cord was transected at the lumbar level in 14 rats. Adrenalin was injected 48 hours later and the rats were sacrificed 24 hours after the adrenalin injection.

Histological examination proved that all these rats had adrenalin myocarditis, in varying degrees of severity just as is seen in the usual control material (unoperated rats after adrenalin injections).

After such an operation cardiac rhythm and ECG remain unchanged. After the adrenalin is introduced the ECG reflects the changes characteristic of the oncoming myocarditis (voltage changes in the waves, alterations in S-T interval and inversions of the T wave).

These results demonstrate the decisive role played by the level at which the spinal section is performed and the significance of the spinal shock in causing cardiac alterations and the appearance of adrenalin myocarditis.

With the goal of increasing the reflex sensitivity of the spinal centers, 20 rats were decorticated-partial removal of the cerebral cortex from the cerebral hemispheres. In some of these rats immediately after the surgery epileptiform convulsive seizures were observed. All the rats during the entire post-operative period had marked-motor stimulation.

Adrenalin (0.5 cc) was introduced on the 9th post-operative day and the rats were sacrificed 24 hours after the injection. Adrenalin myocarditis was present in 18 rats, in the majority of them, very marked. Repeated ECG's done after the operation showed that pulse fluctuations in the operated rats were minimal and the ECG changes were different from those seen after spinal cord transections. Basically, the wave voltages became somewhat altered, sometimes a deep S wave being seen. The S-T interval and the T wave seemed stable, which leads us to suppose that decortication did not carry in its train profound metabolic alterations in the myocardium.

Putting together the results of this experiment with the experiments in which the spinal cord was transected at the cervical and lumbar levels, we believe that the whole complex of alterations evoked by decortication permitted an intensification of the adrenalin myocarditis by reason of a release of cortical inhibition of the reflex sensitivity of the spinal cord, in part, of course, including the cardiac centers.

Also, it may be that the absence of any basic upset in myocardial metabolism after cerebral decortication (as far as can be judged by the ECG) is another pre-condition for the myocarditis developing after the adrenalin injection.

The data obtained need further physiological and pharmacological analysis. In part it is important to study how spinal shock affects the action of other substances capable of producing myocarditis.

However, a summary of the work already done permits some conclusions.

When the spinal cord is transected above the zone of the cardiac spinal centers (at level C_8–T_1), the rats develop bradycardia and ECG changes testifying to changes in contractility and the metabolic myocardial processes. Histologic studies of such rat hearts revealed myocardial hyperemia and albuminous dystrophy of the muscle fibers.

Introduction of adrenalin into the operated rats does not cause further qualitative ECG changes and an adrenalin myocarditis either does not develop at all or is minimal. This is associated with the functional upset of the cardiac sympathetic innervation, the spinal cardiac centers being within the zone of shock.

When lumbar level spinal cord transection is done, the cardiac sympathetic centers are above the shock level. The cardiac rhythm and ECG remain unaffected. Introduction of the same doses of adrenalin produces in the operated rats an adrenalin myocarditis which gives the characteristic ECG changes.

The extent of the adrenalin myocarditis is variable and comparable to that seen in the controls.

After decortication cardiac rhythm likewise is undisturbed and the ECG changes are inconstant and have a different character from that seen after transection of the spinal cord at the C_8–T_1 level.

It must be assumed that cardiac contractility and myocardial metabolic processes are basically unaffected by such an operation.

Adrenalin introduced after such surgery causes an especially intense development of a myocarditis. This may be due to the heightened irritability of the subcortical centers and the spinal cord in these decorticated animals.

The experiments presented above seem to confirm the fundamental role played by the nervous system in the development of an adrenalin myocarditis. In part, the state of functional condition of the spinal cord cardiac centers seems to be important. From this it seems logical to conclude that the variations seen in the adrenalin myocarditis produced in rats after a single injection of adrenalin must be associated with individual variations in the reflex reactions of the animals.

SUMMARY

Adrenalin myocarditis was studied in rats. The effects of spinal cord transections above and below the spinal cord cardiac centers were studied. Transection at C_8–T_1 level produced spinal cord shock below that level, knocking out the cardiac sympathetic centers. Adrenalin myocarditis in this group of rats was either very mild or absent.

Transection below the centers (at lumbar level) did not give results differing materially from the group in which the myocarditis was induced by the usual single adrenalin injection. This seems to be because the spinal cord cardiac sympathetic centers remained unaffected by the transection.

Finally, decortication seemed to intensify the adrenalin myocarditis; probably because of the release of the central inhibition of the spinal cardiac sympathetic centers.

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NERVOUS CONTROL OF BLOOD-GAS CHANGES FOLLOWING FOCAL PULMONARY LESIONS

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It has been shown in many experiments, chiefly clinical, that there is very little relationship between the extent of pulmonary damage and the gaseous blood changes resulting from it. As a rule, the oxygen value of the arterial blood returns to its usual level before recovery from the pulmonary damage has been effected [7, 8, 9, 11].

In previous experiments [2, 3], we have shown the importance of the nervous system in compensating respiratory disturbances caused by pulmonary damage. In these experiments, respiratory function was studied chiefly by recording respiratory movements. A blood-gas analysis was performed only in isolated cases. Nevertheless, the blood gases constitute one of the most important indices of respiratory function.

In the present work we have investigated the part played by the nervous system in compensating blood-gas changes following focal pulmonary injury.

EXPERIMENTAL METHODS

The experiments were carried out on unanesthetized rabbits. In all, 45 animals were used in the experiments. They were placed on their backs and fixed to a support. The damage was effected by introducing 3 ml of water heated to 80-90°C through the chest wall. The advantage of this method is that the damage is immediately effective and there is no incubation period as there is in the case of bacterial pneumonia, or when irritant substances are introduced through the respiratory passages, and there is no possibility of any direct action on the respiratory centers. Samples of arterial blood were obtained from the femoral artery, and venous blood was obtained from the right heart. The blood from the heart was obtained by means of a catheter introduced through the jugular vein. The blood was collected under oil. Blood-gas analysis was carried out using Van Slyke's apparatus, not later than 30 minutes after drawing the blood.

At the beginning of the experiment, the femoral artery and jugular vein were exposed under local anesthesia. After thirty minutes, the first samples of blood were taken. Thirty minutes after the blood had been taken, the lung damage was inflicted and a sample of blood was taken immediately. The next portion of blood was taken 20 to 60 minutes later, and again, as a rule, two to three hours after infliction of the lung damage. A record of the respiration rate was also taken.

EXPERIMENTAL RESULTS

The oxygen saturation of arterial blood has an average value of 95%. Incomplete oxygen saturation of the arterial blood would normally be ascribed to a physiological atelectasis of the pulmonary tissue.

As the experiments lasted 4-5 hours, and as the collections of blood caused a certain anemia, control experiments were carried out to find what effect these factors had on the blood gases. It was found that the effect was quite insignificant, there being only a small reduction in the venous oxygen content, as a result of which the arterio-venous difference increased by 1-2 vol.%.

Blood-gas changes following localized pulmonary damage in rabbits with no damage to nervous system. In these experiments 30 rabbits were used. Blood-gas analysis (carried out immediately after the damage had been inflicted) gave the following results. The oxygen content of arterial blood was reduced by 1.2-3.2 vol. %. The oxygen saturation of arterial blood was reduced by 5.5-15.6%; the CO_2 content was reduced by 1.8-3.8 vol. %.

In the venous blood, the oxygen content was reduced by 2.2-4 vol. %. The CO_2 content was reduced by 2-4 vol. %.

The arterio-venous difference and volume remained within normal limits.

After 20-60 minutes from the time of inflicting the pulmonary damage, the arterial blood was 4-13% below complete saturation. After 2-3 hours, the oxygen saturation, with only a few exceptions, returned to normal. The oxygen content of venous blood remained 1.2-2.9 vol. % below normal, and this caused an increase in the arterio-venous difference of 0.5-2.1 vol. %. The CO_2 content in both arterial and venous blood was reduced by 2-5 vol. %.

Previous experiments have shown that localized pulmonary damage in rabbits, caused by the same methods used in the present experiments, led to an increase in the frequency and depth of respiration for the first 15-20 minutes, after which the respiratory movements returned to normal. We found the same thing. We also found that at the same time there is a hypoxia. This hypoxia disappears 2-3 hours after the infliction of the damage, at a time when the injury not only has not healed, but in fact is continuing to develop.

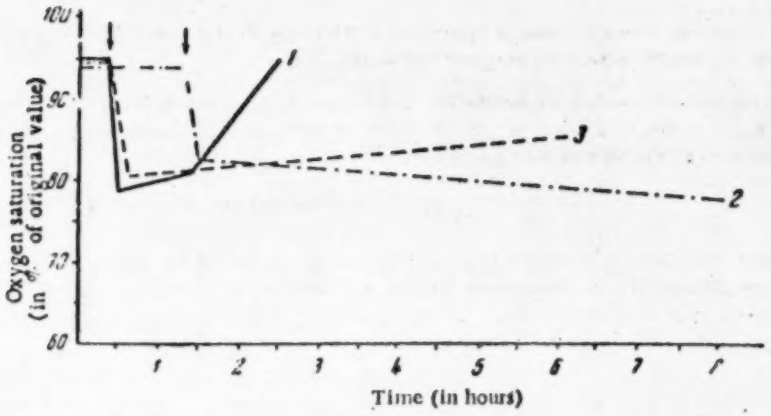
Thus, the present experiments show that the degree of oxygen saturation of arterial blood (see diagram), which is normally taken as one of the principal indices of respiratory function, returns to its normal value before repair of the pulmonary injury has taken place. Postmortem examination carried out after the restoration of respiratory function revealed hemorrhages and edema in the damaged portion, and sometimes regions of atelectasis.

Blood-gas changes following localized pulmonary damage in vagotomized rabbits. In previous experiments in this laboratory [2], we have shown the great importance of the vagi in compensating respiratory damage following localized pulmonary injury. The present investigation of the blood gases in these animals demonstrates this compensatory process particularly clearly.

In these experiments 10 animals were used. A determination of the blood gases was first made in the normal animal, and then repeated, first 30 minutes after resection of both vagi in the neck, then immediately after infliction of the pulmonary damage (which was effected one hour after cutting the vagi), and then 2, 6, and, in some cases, 24 hours later. Section of the vagi in the neck was carried out very rapidly under local anesthesia. After the operation, the breathing became slower and deeper. There was an increase in muscle and facial muscle tone. Thirty minutes after sectioning of the vagi, the breathing became quieter, more regular, and rather more rapid. There was usually no change in the blood gases. These observations agree with other published data [6, 10]. However, it was found that the power to compensate for localized pulmonary damage was considerably reduced in vagotomized animals.

After the pulmonary damage had been effected, the breathing again became deep and labored, but the frequency remained practically unchanged. This agrees with previously published observations [2]. In the arterial blood, the oxygen content was reduced by 1-3 vol. %, and the oxygen saturation was reduced by 5-12%. The oxygen capacity remained within normal limits. In the venous blood, the oxygen content was reduced by 2-3.5 vol. %, and this caused an increase in the arterio-venous difference of 0.5-2 vol. %. The CO_2 content in both arterial and venous blood was reduced by 2-5 vol. %.

Blood-gas analyses carried out after 2, 6, and 24 hours, revealed considerable hypoxia. The oxygen saturation of arterial blood was reduced by 5-16% (see figure). Besides the hypoxia, there was a considerable hypocapnia; the CO_2 content of the arterial blood was reduced by 5-8 vol. %, while the arterio-venous difference



Changes in the degree of oxygen saturation of arterial blood (in % of original value) after damage to lungs by scalding: (1) in animals without interference to nervous system; (2) in vagotomized animals; (3) in animals with impaired nervous trophic functions. The arrows show the times at which the lung damage was effected.

was increased by 1-2 vol. %. The animals died after 24 hours. Postmortem examination revealed a pulmonary edema which was particularly marked on the damaged side.

Investigation of blood gases following localized pulmonary damage in rabbits with impaired nervous trophic function. In the control animals of the first experiments, the blood gases returned, as a rule, to their normal value 2 to 3 hours after the pulmonary damage had been effected. Certain animals which were an exception to this rule were very carefully investigated. We noticed that two rabbits in whom the blood gases did not return to their normal value within 5 to 6 hours showed effects of impaired trophic function in the limbs.

A. D. Speransky [5] and his co-workers [1, 4] showed that a dystrophy of this type is not a localized phenomenon, but reflects a general disturbance of nervous trophic function.

It seemed reasonable to suppose that in animals with signs of dystrophy the failure to effect compensation was due to a generalized impairment of trophic function. We therefore started another experiment to investigate compensation of respiratory function in animals with experimentally induced impairment of nervous trophic function. The simplest form of impairment consists of section of the sciatic nerve.

Five animals were used for this experiment. The sciatic nerve was cut, under local anesthesia, in the upper third of the thigh. After two to three weeks, ulcers developed on the foot.

The blood gases of these animals, determined before and immediately after effecting the pulmonary damage, were within normal limits. However, the oxygen saturation of the arterial blood of these animals did not return to normal within 6 hours. The blood remained 7.4-10.1% below saturation, while in the intact animals the oxygen saturation returned to normal within 2-3 hours after infliction of the pulmonary damage (see figure).

These results show that the hypoxia cannot be explained in the terms of the failure of the oxygen to diffuse into the damaged zone. This follows from the fact that the oxygen saturation of the blood in animals with an intact nervous system is restored to its normal value before the pulmonary damage has been repaired.

It is clear that the oxygen saturation of the blood does not depend directly on the change in respiratory movements or on the pulmonary ventilation. After the pulmonary damage has been inflicted, the breathlessness continues for 15-20 minutes, while the hypoxia lasts for 2-3 hours.

We considered above the possibility that the hypoxia is due to a small part of the blood continuing to circulate through the damaged portion of the lung without becoming oxygenated. We must also suppose that in these present experiments the hypoxia arose in this way.

Lung damage in normal animals caused a hypoxia of a relatively short duration of 2-3 hours, while in vagotomized animals the condition became progressively worse.

In addition to the nervous mechanism controlling pulmonary ventilation and blood circulation through the lungs, the general trophic action of the nervous system is also involved to a considerable extent. As our experiments have shown, elimination of this trophic function hinders the development of compensatory changes.

SUMMARY

In focal injury of lung tissue in experiments on rabbits, the restoration of the gaseous content of the blood takes place when the pathological foci are not only present, but are even in a condition of development. The restoration of the normal content of blood gases is due to the change in the relationship between the ventilation and circulation of the lungs. Resection of the vagus nerves hinders this mechanism. Due to that, injury of the lungs in animals with resected vagus nerves induces progressive hypoxemia. Disturbance of the nerve trophics, likewise reduces the ability of the organism to compensate the disturbance of respiration, caused by focal injury of the lungs.

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RESTORATION OF HIGHER NERVOUS ACTIVITY IN DOGS RESUSCITATED AFTER 30-MINUTE CLINICAL DEATH IN CONDITIONS OF HYPOTHERMIA

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Many authors [1-8] have studied the disturbance to the highest sections of the brain after different periods of clinical death caused by acute blood loss and the extent of their functional restoration. T. S. Fedotov [6], V. D. Yankovsky [7], N. N. Sirotinin [5], L. I. Mursky and V. K. Ustinova [2] and others conducted experiments under ordinary conditions of temperature. Recently, the duration of clinical death has been successfully lengthened to 1 hour [4] by means of hypothermia in the animals suffering the acute blood loss. It is still not clear how much the higher sections of the central nervous system suffer in dogs resuscitated after lengthy periods of clinical death in conditions of hypothermia. The purpose of this work, therefore, was to determine how higher nervous activity changed in 4 dogs, which were resuscitated after 30-minute clinical death caused by acute blood loss in conditions of general body cooling.

EXPERIMENTAL METHODS

The experiments were conducted on 4 male dogs weighing from 17.5 to 21.5 kg. Before the experiment, the animals were injected with a 2% solution of pantopon, in a dose of 2 ml per 5 kg of weight and with 1 ml of a 0.1% atropine solution. Body temperature was reduced by physical cooling combined with an intravenous injection of a 0.2% pentothal solution. Clinical death was caused by massive blood-letting from the femoral artery. The vital functions were restored in the animals by means of a complex resuscitation method developed by V. A. Negovsky. Fibrillation, when it occurred, was eliminated by single condenser discharges (according to N. L. Gurvich's method). After cardiac activity had been restored, the animals were warmed by immersion in warm water (43-45°). In order to determine the extent to which the functions of the cerebral cortex had been restored in the resuscitated animals, a system of conditioned reflexes had been previously developed according to a stereotype consisting of 5 positive stimuli (metronome 120, light, bell, light, metronome 120) and one inhibitory stimulus (metronome 60). The acid-defense method was used in these experiments. An 0.3% solution of hydrochloric acid was used as the unconditioned stimulus.

EXPERIMENTAL RESULTS

The animals were bled when their body temperature was from 25.8° to 26°. Clinical death lasted 30 minutes. The dying period on a background of cooling lasted from 9 minutes, 35 seconds to 26 minutes. At the time of resuscitation, body temperature in the animals was reduced to 23.8-25°. Heart action in the dogs was restored after intervals of from 1 minute, 13 seconds to 4 minutes, 30 seconds. The later heart action restoration was caused by the appearance of fibrillation during the first stage of resuscitation. Respiration was restored after intervals of from 7 minutes, 23 seconds to 58 minutes, 55 seconds. The corneal reflex was restored 35 minutes, 30 seconds to 46 minutes, 25 seconds after the beginning of resuscitation. The animals were warmed for 1 hour,

30 minutes from the beginning of resuscitation; after this warming period, the body temperature was 31-32°, and after 13-15 hours, it was as high as 37.2-37.6°. The animals were in a somnolent condition during the first 9-14 hours after resuscitation. Their vision and hearing were restored after 9½-16 hours, and after 16-21 hours, they began to walk and to take food independently. The animals' condition was completely satisfactory by the end of the first day after resuscitation was begun; by the 2nd day, their outward behavior was approximately the same as that of healthy animals. Before the experiment, the first conditioned reflex reaction to the metronome 120 appeared after the 7th combination in the dog August, to the light, from the first combination, and to the bell, after the 7th combination. The differentiation began to be developed after the 7th use of the inhibitory stimulus. The state of the conditioned reflexes in the dog August before resuscitation is given in the report of Experiment No. 85. Sixteen hours and forty-five minutes after resuscitation, August was taken into the conditioned reflex chamber. The data obtained from the first three experiments after resuscitation show that higher nervous activity was disturbed in these dogs, although there were no noticeable changes in their general behavior.

Note the low level of the conditioned reflexes in the first experiment after resuscitation (Experiment No. 1).

No. Experiment and date	Time exp. (in hrs. and min)	Ordinal No conditioned stimulus	Conditioned stimulus	Time conditioned stimulus acted alone (in sec.)	Magnitude conditioned reflexes (No. drops) total	Magnitude conditioned reflexes (No. drops) for each 5 sec.	Latent period conditioned reflexes
Before clinical death							
85 May 23,1955	12:25	296	M_{120}	20	1	1–0–2–1	5
	12:28	81	Light	20	3	2–0–0–1	3
	12:31	89	M_{60}	30	–	–	–
	12:34	92	Bell	20	3	0–1–1–1	9
	12:37	82	Light	20	2	1–0–0–1	4
	12:40	237	M_{120}	20	3	0–1–1–1	7
After resuscitation							
1 May 25,1955	10:25	1	M_{120}	20	1	0 0–1–0	14
	10:28	1	Light	20	–	–	–
	10:31	1	M_{60}	30	1	0 0 1–0–0–0	–
	10:34	1	Bell	20	–	–	–
	10:37	Saliva funnel torn off				–	–
2 May 25,1955	15:40	2	M_{120}	20	1	0–0–0–1	18
	15:43	2	Light	20	8	2–6–0–0	4
	15:46	2	M_{60}	30	5	4–1–0–0–0–0	–
	15:49	2	Bell	20	–	–	–
	15:52	3	Light	20	12	2 0–4–6	4
	15:55	Funnel torn off					
3 May 27,1955	15:45	3	M_{120}	20	1	0 1–0–0	8
	15:48	4	Light	20	1	0–1–0–0	7
	15:51	3	M_{60}	30	34	3–13–12–6–0–0	–
	15:54	3	Bell	20	–	–	–
	15:57	5	Light	20	3	0–0–3–0	14
	16:00	4	M_{120}	20	–	–	–
17 June 16,1955	15:20	34	M_{120}	20	7	1–3–3–0	5
	15:23	36	Light	20	3	0–1–1–1	9
	15:26	16	M_{60}	30			–
	15:29	16	Bell	20	2	0–0–1–1	14
	15:32	37	Light	20	4	0–1–1–2	5
	15:35	35	M_{120}	20	3	0 1–1–1	8

After the bell, the dog manifested a strong motor excitation, during which he tore off the saliva funnel.

Forty hours after resuscitation (Experiment No. 2), the animal was somewhat calmer during the experiment; the conditioned reflex stereotype, however, was not yet completely restored. The most noteworthy fact was the paradoxical behavior of the nervous system in response to stimuli which had caused an adequate reaction before the clinical death. On the third day (Experiment No. 3), the character of conditioned reflex activity was approximately the same as on the second day, but the appearance of an ultraparadoxical phase was observed.

On the 4th day after resuscitation (Experiment No. 4), a single eradication of the conditioned reflexes to the metronome 120 was done.* This caused conditioned reflex activity to improve; the tonicity of the cerebral cortex cells was raised, and the conditioned reflexes began to become stable. Bromine was used during the 5-day rest period after the 12th experiment (on the 17th day after resuscitation) in order to improve the correlation of the principal nervous processes. The animal received 0.6 g of bromine with milk on the first day and 1.8 g, fed twice a day, on the following days. The results obtained from the use of bromine are shown in the Table (Experiment No. 17). Therefore, the use of bromine gave positive results. The differentiation was strengthened, and the character of conditioned reflex activity became almost normal. Of course, the normalization of conditioned reflex activity was connected with the time which had elapsed since the moment of resuscitation as well as with the bromine treatment.

A similar picture of activity restoration in the higher sections of the brain after 30-minute clinical death in conditions of hypothermia was observed in the other three experimental animals – Baikal, Ruslan and Treasure. Differences were observed, however, during the beginning of restoration (hypnotic phases absent) and during the administration of the bromine preparations, which had to be used in all of the dogs. This much is clear: that the more vulnerable process of inherent inhibition is the process most weakened by conditions of clinical death. That the stimulation process was also considerably weakened was shown by the fact that experiments in the conditioned reflex chamber could not be done daily, since this caused the progressive exhaustion of the cerebral cortex cells. Our dogs had very different types of higher nervous activity. This is the reason why, in the experiments using sodium bromide, Baikal (weak variant of the strong type) was given sodium bromide, for 4 days in doses of 0.5-0.6 g, while Ruslan, a strong, balanced, mobile type, received an average of 3 g of sodium bromide daily for 6 days, and Treasure, which had an extremely weak type of higher nervous activity, was given 0.1 g of sodium bromide daily for 20 days.

All of the experimental dogs lived. Their higher nervous activity was completely restored 13-23 days after the start of resuscitation.

Therefore, our study of higher nervous activity restoration in dogs after 30-minute clinical death caused by lethal blood-letting under conditions of hypothermia showed that the conditioned reflexes could be completely restored in the dogs; there were, however, different disturbances of the relationships between the principal nervous processes in the different animals during their restitution. These disturbances were either manifested by extremely acute functional exhaustion in the higher sections of the central nervous system or by a hypnotic condition expressed to varying degrees in the animals from slight, compensating relationships to extremely pronounced forms of an ultraparadoxical phase.

SUMMARY

The characteristic features of recovery of the function of the brain cortex after resuscitation was studied in 4 dogs. For that purpose a system of conditioned reflexes was previously developed. These reflexes were formed according to a stereotype, consisting of 5 positive (metronome – 120 light, bell, metronome – 120) and one inhibitory (metronome –60) stimulants. The acid-defense method was used in these experiments. The possibility of complete recovery of the previously developed conditioned reflexes after a 30-minute clinical death, caused by acute blood loss in condition of hypothermia was proved in these animals.

Various disturbances of relationship between the fundamental nervous processes took place during the process of recovery.

These disturbances were either in the form of extreme exhaustion of the functions of the highest portions of the central nervous system, or gave the picture of various degree hypnotic condition.

*Our experiments have shown that this method often strengthens the process of inherent inhibition.

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THE EFFECT OF THE THYROID GLAND ON THE MORTALITY RATE IN SHOCK-HEMORRHAGIC CONDITIONS CAUSED BY STRONG SOUND STIMULI

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In a previous work [4], we described the methods used and the results effected by the action of a sound stimulus (electric bell) in the development of serious shock conditions in rats. The studies showed that after 15-18 minute action of a sound stimulus causing acute excitation in the animal, the animal began to manifest signs of a serious shock condition: disturbance of motor coordination, paresis of the extremities, nystagmus, etc. In 12.7% of the animals, the shock condition caused death. When the animals were dissected, cerebral hemorrhages were found, caused by the outward passage of the blood from the vascular bed due to diapedesis.

The leading factor in the development of shock-hemorrhagic conditions was the acutely stimulated state of the brain, caused by the action of the sound stimulus. The death of the animals was usually not observed so long as the extent of stimulation was limited by the restraining protective inhibition. Death occurred after the inhibitory-protective function of the nerve cells had weakened. In our experiments, this condition was obtained by means of a three-minute rest period after 15-minute interrupted sound stimulation. The use of the strong stimulus again, after the rest period, caused a sharp outburst of excitation no longer restrained by the protective inhibition, which had weakened during the three-minute interval, and this led to a serious shock condition ending in death.

The initial level of nervous system excitability in the experimental animals materially affects the mortality rate in nervous traumatism; due to a previous injection of caffeine (10-20 mg per 100 g of rat's weight), for example, the mortality rate increased considerably.

The internal secretion glands influence greatly the development of shock-hemorrhagic conditions. Twice as many males as females died as a result of the nervous trauma. Castration of the males decreased the percentage of death from shock-hemorrhagic conditions, but removal of the parathyroid glands considerably increased it.

This work presents the results we obtained when studying the role of the thyroid glands in the mortality rate of rats with shock-hemorrhagic conditions caused by the action of strong sound stimuli.

EXPERIMENTAL METHODS

The work was done on rats sensitive to a sound stimulus; the rats were, for the most part, males. The rats were subjected to the action of a continous sound stimulus for $1\frac{1}{2}$ minutes and then to the action of an interrupted sound stimulus with 10-second sound exposures alternating with 10-second intervals for a period of 15 minutes. Then came a 3-minute rest period, after which the strong sound stimulus was used again.

The experiments were conducted using either hyperthyroidism or extirpation of the thyroid glands.

Thyroidin was administered to the rats (weighing from 150 to 200 g) per os in doses of from 0.025 to 0.1 g for a period of 10-14 days. After this, the animals were subjected to the traumatic action of the sound stimulus by the method already described. Rats of the same sex and age which had not received thyroidin were used as the control.

The thyroid and parathyroid glands were extirpated. In the control group of rats, only the parathyroid glands were extirpated. Both the experimental and control rats of this group were subjected to the action of the sound stimulus 10-15 days after the operation.

EXPERIMENTAL RESULTS

Table 1 shows the effect of hyperthyroidism on the mortality rate of the rats which were subjected to the traumatic action of the sound stimulus.

Table 1 shows the extraordinarily large influence of hyperthyroidism on death in the rats with the induced nervous trauma. The mortality of the hyperthyroid rats was 8 times higher than that of the control rats. One should also mention that the shock condition was far more serious in all the hyperthyroid animals than in the control both during the action of the sound and after it. From the first minutes of the sound stimulus action, signs of a serious shock condition developed in most of the hyperthyroid rats, rapidly terminating in the death of the animal. Macroscopic examination of the dead experimental and control rats showed no essential differences in the frequency of cerebral hemorrhages.

TABLE 1

The Effect of Hyperthyroidism on the Mortality Rate in Nervous Trauma

Group of Animals	No. rats in exp.	Hemorrhages found		
		in absolute No.	in %	
Hyperthyroid	103	69	66.6	78.6
Control.....	108	9	8.3	83.3

* Forty-two of the 69 dead hyperthyroid rats were dissected, and 6 of the 9 control rats were dissected.

TABLE 2

The Effect of Thyroidectomy on the Mortality Rate in Nervous Trauma

Type of Operation	No. of rats in exp.	of which died		
		in absolute No.	In %	Hemorrhages found
Thyroparathyroidectomy (experiment)	35	9	25.7	44.4
Parathyroidectomy (control)	42	18	42.9	75.0

* Nine rats were dissected in the experimental group, 16 in the control.

Table 2 shows how removal of the thyroid glands affected the mortality rate of the animals subjected to nervous trauma.

Table 2 shows that the number of deaths caused by nervous trauma was much smaller after extirpation of the thyroid and parathyroid glands than after parathyroidectomy alone. Statistical evaluation of the material obtained showed that the difference in the mortality rate of the animals in the indicated groups exceeded the probable error 2.31 times. Although the authenticated difference is not great, the data obtained allow the proposal that thyroidectomy has a tendency to decrease the possibility of death resulting from the shock conditions.

Macroscopically, fewer cerebral hemorrhages were found in the experimental animals than in the control. The data presented show that removal of the thyroid glands decreases the possibility of death from brain hemorrhages in animals as a result of a shock condition brought on by a strong sound stimulus.

One must mention that the decreased mortality effect of the thyroidectomy in the experiment described appeared on a background of heightened sensibility of the animals to shock conditions, which state was observed after parathyroidectomy.

Therefore, our experiments showed that change in thyroid gland activity materially affects the mortality rate in shock conditions: hyperthyroidism sharply increases the mortality rate, while a thyroid condition, on the other hand, somewhat lowers the mortality rate.

These effects are possibly connected with the influence of the thyroid gland hormone on the tonicity and permeability of the vascular wall and on the blood pressure level [2, 3, 4, 5, 6].

SUMMARY

In prolonged (15-18 minute) action of sound stimulant, a condition of shock is developed in rats after the stage of pronounced excitation. In 12-15% of cases it is terminated by death. Brain hemorrhages are found at autopsy, which are the result of diapedesis. The fatal outcome in shock-hemorrhagic conditions depends on the relationship of the processes of excitation and inhibition and the functional condition of the endocrine glands. The functional level of the thyroid gland has the most pronounced influence on the fatal outcome in shock-hemorrhagic conditions. In animals with hyperfunction of the thyroid gland mortality rate reaches 66.6% (with only 8.3% in control animals). In extirpation of the thyroid gland the mortality rate is reduced in comparison with control animals (it is 1.7 times higher in control animals).

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EXPERIMENTAL DATA ON THE THERAPEUTIC EFFECT OF PARA-AMINO-SALICYLIC ACID (PAS)

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Although PAS is widely used for tubercular diseases, the mechanism of its effect has not been sufficiently studied. More particularly, the question of PAS's effect on the nervous system and the importance of this effect to the mechanism of the preparation's therapeutic action has only been touched upon. With this in mind, we decided to study the effect of PAS on the interoceptive reflexes.

In previous works conducted on healthy cats[6, 7], we established that PAS causes stimulation of the chemoreceptors by indirect contact with them. In the second phase of its action, the preparation causes a reversible functional interruption or a considerable decrease in chemoreceptor excitability. It was also established that PAS injected intramuscularly inhibits reflexes from the chemoreceptors, while increasingthe reflexes from the mechanoreceptors in a majority of experiments.

In this work, we present results obtained in experiments studying the effect of PAS (0.25-1.0 per 1 kg of weight) on reflexes caused by stimulating the chemoreceptors of the small intestine, the receptors of the carotid sinus reflexogenic zone and the bladder mechanoreceptors in animals suffering from tuberculosis.

EXPERIMENTAL METHODS

A total of 19 animals were infected with an emulsion of bovine tuberculosis microbes (K-2 strain). The culture was injected into the femoral vein in a dose of 0.025-0.02 mg per 1 kg of weight.

The animals were used in the experiment 10-25 days after the infection, after which they were subjected to careful pathologico-anatomical examination. Tuberculosis was observed in all of the animals beginning the 14th day after infection, and becoming most strongly expressed on the 20th-25th day after infection.

The arterial pressure and respiratory reflexes from the chemoreceptors of the small intestine were examined under conditions of perfusion of the organ, which was isolated from circulation. We used acetylcholine in a concentration of $1 \cdot 10^{-4}$ (1 ml) or carbon dioxide as a stimulant. The reflexes from the carotid sinus reflexogenic zone were elicited by pressure on the common carotid artery for a period of 15 seconds, and the reflexes from the bladder mechanoreceptors, by air expansion of the bladder walls with a pressure of 80-100 mm of mercury.

EXPERIMENTAL RESULTS

In the majority of experiments conducted, PAS caused the reflexes from the chemoreceptors of the small intestine to decrease by 25-75%. In several experiments, the reflexes from the chemoreceptors became inhibited as soon as 15-20 minutes after the PAS injection (Fig. 1).

The reflexes from the receptors of the carotid sinus reflexogenic zone were completely inhibited. In this series of experiments, as opposed to the experiments on healthy animals, the reflexes from the bladder mechano receptors were also inhibited. Only in 3 out of 11 experiments were the reflexes observed to increase. In 6 experiments, the reflexes decreased (Fig. 2), and, in 2 experiments, the reflex remained practically the same. The PAS injection also inhibited the respiratory reflexes in the majority of experiments.

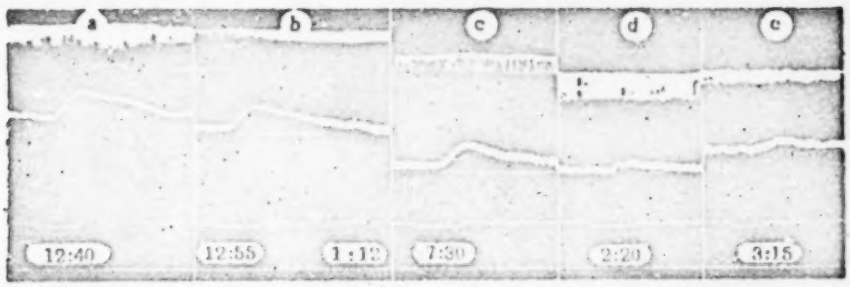

Fig.1. Decrease in reflexes from the chemoreceptors of the small intestine in an animal with tuberculosis after intramuscular injection of PAS (0.5 g per 1 kg of weight).
a, b) initial reflexes; c, d, e) reflexes after PAS injection. Curves from top to bottom signify: respiration, blood pressure, indication of stimulation, indication of time (in 5 second marks).

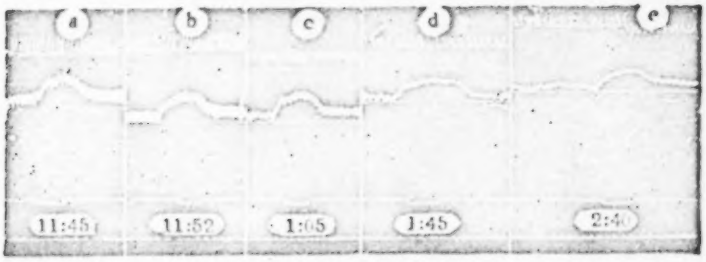

Fig. 2. Decrease in reflexes from bladder mechanoreceptors in an animal with tuberculosis after PAS injection. PAS injected at 12:32 PM.
a, b) initial reflexes; c, d, e) reflexes after PAS injection. Curves mean the same as in Fig. 1.

Having established that PAS, parenterally injected, has a primarily inhibitory effect both in healthy and in tubercular animals on the reflexes from the chemoreceptors, we went on to determine what effect PAS had when inadequate stimulants, causing pathological reactions, were used. We used post-transfusion shock for this study.

It has recently been established [2, 9 and others] that the reflexes from the vascular chemoreceptors, which occur due to the stimulation of these chemoreceptors by heterogeneous blood and by the secondary products resulting from the reaction of the host's blood with the transfused blood, play an important part in the development mechanism of post-transfusion shock.

We caused shock to develop by infusing 10 ml of preserved donor's blood into a femoral vein. The experiments were done on 16 cats. The animals were matched in pairs according to sex and weight and then separated into a control and an experimental group. The animals were intramuscularly injected with PAS in a dose of 0.5-1 g per 1 kg of animal weight 45 minutes to 1 hour, 25 minutes before the experiment.

In all of the control experiments, the infusion of 10 ml of preserved blood into the femoral vein caused

monotypic and rather pronounced disturbances in circulation and respiration. The change in circulation consisted of an abrupt drop of the arterial pressure level, occurring in most cases 20-60 seconds after the foreign blood infusion. In these experiments, arterial pressure fell 39-112 mm, an average of 69 mm of mercury. In the majority of experiments, the lowered blood pressure gradually (over a period of 2-3 or more minutes) returned to the original level.

Respiratory changes were observed simultaneously with the circulatory disturbances. These changes were manifested by a complete inhibition of respiratory movements, interrupted occasionally by single inhalations.

The latent period was 25-60 seconds, and the respiratory movements were inhibited for a period of 20-30 seconds.

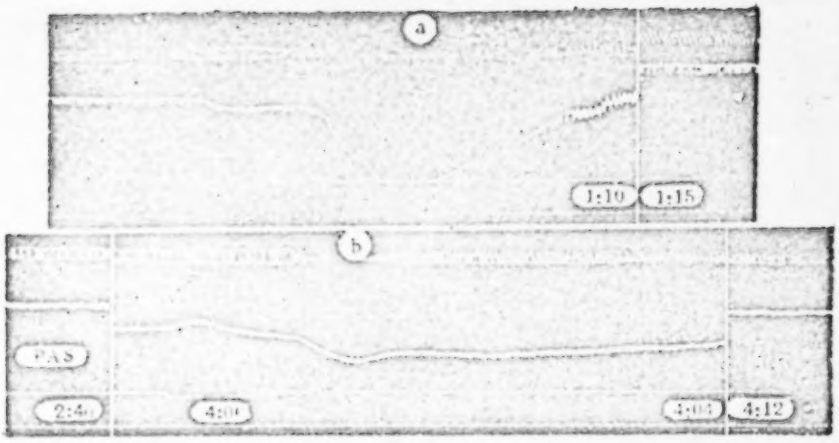

Fig. 3. Effect of PAS on post-transfusion shock.
Results of both the experiment and the control given in the figure: a) control, b) 10 ml of preserved donor's blood infused into the femoral vein 1 hour and 20 minutes after the intramuscular injection of 20 ml of a 10% solution of PAS. The curves are the same as in Fig. 1.

The results obtained from the experiments with the animals given a preliminary injection of PAS 45 minutes to 1 hour and 25 minutes before the transfusion of donor's blood were quite different. Although blood pressure and respiration changes were also observed in these experiments, they were much less expressed than in the control. Arterial pressure decreased less in the experimental animals than in the control animals, which had not been injected with the preparation. In 7 out of 8 experiments, for example, it fell 10-69 mm of mercury, and, in the other experiment, it remained unchanged. In these experiments, arterial pressure fell an average of 46 mm of mercury, or 66.6% of the average arterial pressure fall in the control experiments.

As far as the respiratory reaction was concerned, the complete inhibition of respiration so typical of heterotransfusion shock was absent in 3 out of 8 experiments. Respiration was inhibited in the other 5 experiments, but less than in the control.

Upon comparison of the arterial pressure and respiration reactions caused by the heterogeneous blood transfusion which occurred in the experiment with those which occurred in the control, it is evident that the preliminary injection of PAS reduced the post-transfusion shock in 5 out of 8 experiments (Fig. 3).

From the results of our observations, one can conclude that PAS weakens one phase of post-transfusion shock, which phase may possibly be connected genetically with the participation of the chemoreceptors.

We know from the literature that overstimulation of the interoceptors, and of the chemoreceptors particularly, plays an important role in the development of pathological processes in the body [1, 4, 9 and others]. As has recently been established, in a tuberculosis infection, overstimulation of the chemoreceptors by the activity and decomposition products of the tuberculosis microbacteria as well as by pathological exchange products which form in great quantities at the focus of specific inflammation, is observed [3, 5, 8 and others].

Since PAS inhibits the reflexes from the chemoreceptors, judging from our observations, it must thereby prevent this overstimulation and, therefore, also the appearance of a series of complex reflex phenomena, which are very important to the pathogenesis of the tuberculosis infection and its clinical manifestations.

It is possible that the rapid symptomatic effect obtained from the therapeutic use of PAS in tuberculosis, which usually occurs before positive changes can develop in the anatomical picture of the disease, is due to these established pharmacological properties of PAS.

SUMMARY

Experiments on animals infected with tuberculosis bacilli have shown that parenteral injections of PAS inhibit reflexes from chemoreceptors. The same effect of PAS on interoceptors has been elicited in cases of pathologic reflexes originating from chemoreceptors. The instantaneous symptomatic reaction of tuberculosis patients treated by PAS is possibly due to its inhibitory effect on reflexes from chemoreceptors.

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THE EFFECT OF NOVOCAIN BLOCK ON THE CATALYTIC BREAKDOWN OF NOVOCAIN IN ENDARTERITIS OBLITERANS

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The wide use of novocain as a local anesthetic and therapeutic agent gives rise to many problems requiring investigation.

It has been shown that after intravenous or subcutaneous injections of novocain in animals [5, 8, 9] and man [7, 10] there is a rapid breakdown into paraaminobenzoic acid and diethylaminoethanol. Both these products of hydrolysis are less toxic than novocain itself [6, 19]. The paraaminobenzoic acid is almost completely eliminated from the organism in the urine, 20-30% of the diethylaminoethanol is excreted, and the fate of the remainder is unknown. There is some evidence that it is absorbed by the tissues – mostly by the spleen, and least by the skeletal muscles and blood plasma [19]. The length of time for which it remains in the body is unknown. There is some evidence that its presence increases the toxicity of novocain, lowering the value of the lethal dose by approximately 35% [11, 12, 23].

We have previously shown that the amount of unchanged novocain excreted in the urine depends upon the type of block (whether a paranephric or a nerve block), and that it is less in patients receiving repeated blocks at intervals of one to four weeks. These results indicate a variability in the activity of the novocainesterase in the organism, and show the need for investigation of the variability of the enzyme systems responsible for the breakdown of novocain.

It has been shown recently that novocainesterase is present in all tissues, but that its activity depends upon the particular tissue in which it is found. The greatest novocainesterase activity is found in the liver, while in the spleen and brain it has practically no activity. There is also considerable variation in the activity from one animal to another. The novocainesterase of skeletal muscle is particularly inactive [1, 22].

R. Hazard and his co-workers [13] have studied the activity of novocainesterase in blood serum in vitro in man and in various animals, including birds. These authors consider that the activity of this enzyme in man is such as to cause 80-100% hydrolysis of novocain in a 30-minute incubation period. Rodent blood has a higher activity than this, while canine blood has a very low value.

The long-maintained excretion of unchanged novocain in the urine in dogs [1] is some evidence for the low novocainesterase activity, though this has a higher value than was found by Hazard in his experiments in vitro. Evidently the enzyme is very labile, and the action may depend on whether it is in vitro or in vivo.

R. Hazard [13, 14, 15] considers that the determination of the novocainesterase activity of blood may be used as a diagnostic and prognostic test in several diseases. From a wealth of clinical material, he and others have shown that the novocainesterase activity of blood serum is lowered in conditions affecting the liver and thyroid. A particularly low value is found in cirrhosis and cancer of the liver [16, 20] and the activity is lowered for a considerable time in man after cranial trauma [21].

If, as we may suppose, there are individual differences in the activity of the enzymes responsible for the breakdown of novocain, then its rate of destruction will vary from one individual to another.

It is possible that the death of the patient following local novocain anesthesia described by L. G. Kaganova [2] can be explained in this way, as can the dizziness and vomiting in several patients after injections of novocain.

We have investigated the activity of novocainesterase in the hydrolysis of novocain, as well as that of the enzyme causing the acetylation of paraaminobenzoic acid, which is probably a product of this hydrolysis.

EXPERIMENTAL METHODS

The novocainesterase activity of the blood serum was determined in blood taken from patients before starting any treatment, as well as 4-5 days after the first novocain block; we used the method of R. Hazard [10], as modified by us [1].

The activity of the novocainesterase was determined by mixing 100 γ of novocain with 1 ml of blood serum and finding how much was hydrolyzed during a five-minute incubation period at 37°C; a determination of the amount of novocain hydrolyzed during a 30-minute incubation period was also made, and this gave additional information about the extent to which the novocain was broken down.

A quantitative estimation of the action of the enzyme systems causing the hydrolysis and acetylation of paraaminobenzoic acid in vivo was made by determining the amount of unchanged novocain and free and acetylated paraaminobenzoic acid excreted in the urine after novocain block. The novocain and its hydrolysis products present in both blood and urine were determined by the method previously described [1].

Altogether, investigations were made in 34 human subjects; the experimental group consisted of 18 patients with endarteritis obliterans, and the control group consisted of 16 subjects suffering from various other diseases, including: abscesses, carcinoma of the stomach, pyloric stenosis, cicatrization of the esophagus, duodenal ulcer, tuberculosis of the joints, and deformation of the hip joint. All subjects were of approximately the same age. There are various classifications of the types and phases of endarteritis obliterans. We used two classifications, the first being that used in the A. V. Vishnevsky Surgical Institute, and the second that proposed by I. G. Rufanov.

In the first classification, the subjects were divided as follows: 7 subjects (Group I) with atonic, 6 subjects (Group II) with spastic, and 5 subjects (Group III) with mixed forms of the disease.

In the second classification, the subjects were divided as follows: the second period of disease – 5 subjects (Group IV), third period – 9 subjects (Group V).

Patients in whom a sympathectomy had been performed were referred to a separate group, Group VI.

EXPERIMENTAL RESULTS

The percentage amounts of novocain hydrolized in the five-minute incubation period in the case of patients with endarteritis obliterans were: for Group I, 23-56%; for Group II, 28-61%; and for Group III, 42-55%.

These results demonstrate the individual differences in the activity of novocainesterase. The average values for the results for activity changes in endarteritis obliterans patients are shown in Fig. 1.

It can be seen from these results that in all cases the activity of the novocainesterase of the blood serum is below normal. The amount of novocainesterase broken down during a 30-minute incubation period is below normal in half of the cases. In all patients with endarteritis obliterans, the activity of the novocainesterase of the blood system shows scarcely any reduction after novocain block. Normally, injections of novocain increase the novocainesterase activity of blood serum, and this was observed in the control group.

We also found the length of time for which novocain and its hydrolysis products were excreted in the urine after novocain block (Figs. 2 and 3).

In patients with endarteritis obliterans, the unchanged novocain is excreted in the urine for longer periods than in the control group. In patients in Groups I and V, the excretion of unchanged novocain in the urine continues as long as eight hours after the block (Fig. 2, a, b), but does not exceed the amount excreted in the control group (Fig. 2, c). This indicates a reduced rate of hydrolysis of novocain in the body, and agrees with the results found for novocainesterase in blood serum in vitro.

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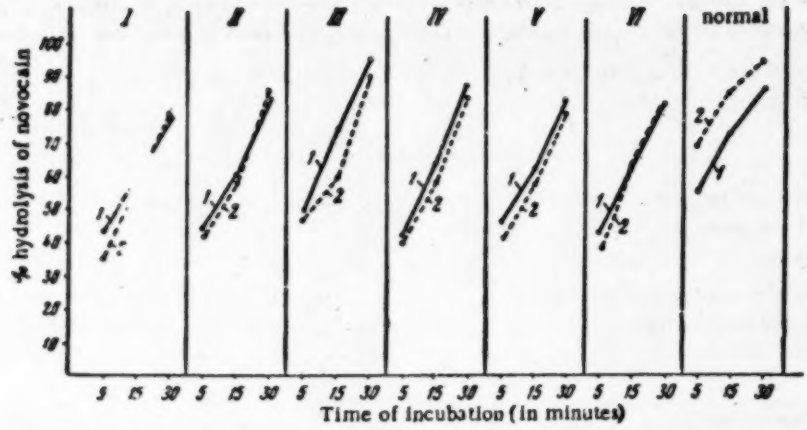

Fig. 1. The effect of novocain block on the activity of novocainesterase blood serum in patients with endarteritis obliterans. Groups I to VI patients with endarteritis obliterans. 1) before block, 2) after block.

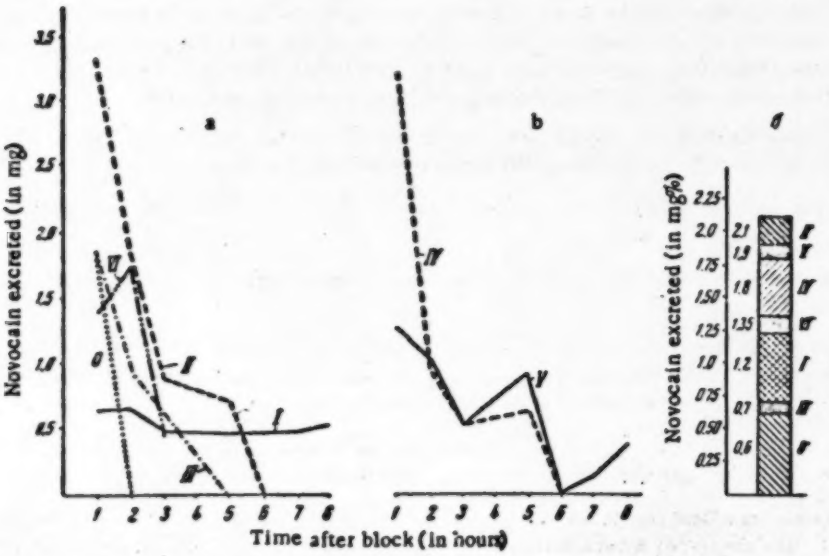

Fig. 2. Rate of excretion of unchanged novocain from the urine after block in patients with endarteritis obliterans, patients of Groups I-VI.
0) Control group; a) groups of endarteritis obliterans patients according to the first classification; b) groups of patients according to the second classification; c) amount of novocain excreted during the whole time after the block.

The results for the atonic forms of the disease must be noted (Group I). The rate of excretion of unchanged novocain is constant for a considerable time, as though a novocain renal threshold had been reached which was independent of the novocain concentration in the body. This effect is clearly associated with changes in the

permeability of the blood vessels. In patients with spastic and mixed forms of the disease (Groups II and III), most of the unchanged novocain is excreted in the first two hours after the block when its concentration in the body is highest; after this the excretion rate becomes considerably reduced, though it continues longer than in patients of the control group. In these, the unchanged novocain is excreted only for one hour after the block, and in very small quantities.

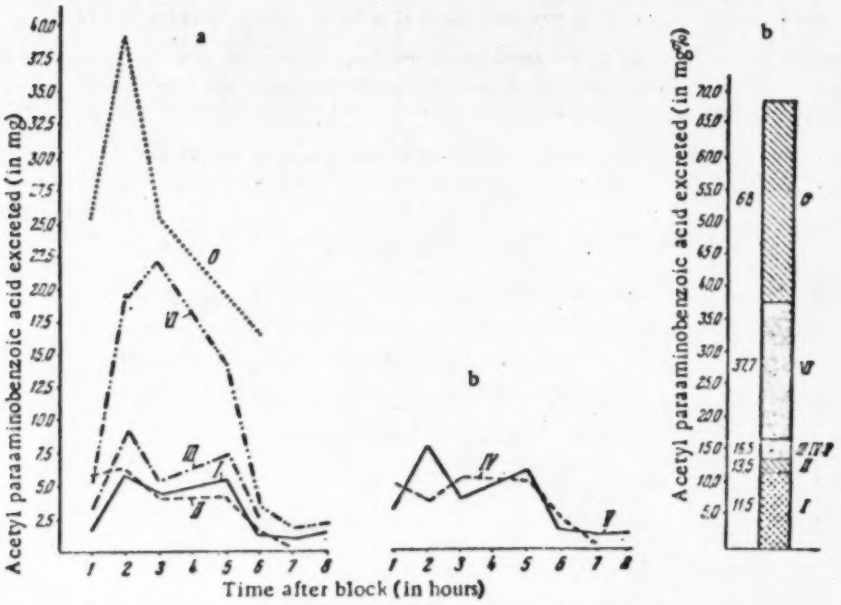

Fig. 3. Excretion of acetyl paraaminobenzoic acid from the urine after novocain block in patients with endarteritis obliterans (legend as in Fig. 2).

Figur 2, c shows details of the excretion of unchanged novocain in the urine for the whole period after the block. The smallest quantity is excreted in the control group, and the greatest amount in patients with the spastic form of endarteritis obliterans (Group II), but in patients with the atonic form of the disease, a smaller percentage of the novocain is excreted, since the investigation was limited to eight hours after the block, and the excretion of unchanged novocain not only had not finished by this time, but the concentration in the urine had not even begun to fall.

Paraaminobenzoic acid, one of the hydrolysis products of novocain, is excreted in the urine of patients with endarteritis obliterans in 2-3 hours, while in the control group the same amount is excreted in the first hour after the block. The amount of the acid secreted does not exceed 4 mg %. A considerable amount of another novocain breakdown product – acetyl paraaminobenzoic acid – is also excreted in the urine, and most of the novocain is excreted from the body in this form (Fig. 3).

The excretion of acetyl paraaminobenzoic acid in patients of Groups I, V and VI, lasts longer than in the control group. In the first groups, the excretion of the acid continues for eight hours after the block, while in the control group it is complete in six hours (Fig. 3, a, b). In all the endarteritis obliterans patients the percentage of acetyl paraaminobenzoic acid excreted is considerably less than normal (Fig. 3, c).

Thus, there are individual differences in the ability to break down novocain, but in endarteritis obliterans patients hydrolytic enzyme activity is reduced, and there is also a considerable reduction in the amount of acetyl paraaminobenzoic acid excreted after the block. This latter effect may be due to a change in the permeability of the blood vessels. However, it could be due to a general reduction in the processes of acetylation in the patients, and this in turn may be associated with a lack of pantothenic acid or coenzyme A. When, as a result

of the hydrolysis of novocain, more than the normal amount of paraaminobenzoic acid is formed, the lack of pantothenic acid may result in a reduced amount of acetylation. This has been shown by T. Riggs and D. Hegsted [17, 18]. Determination of the novocainesterase activity of the blood indicates how a patient will react to novocain, and according to certain investigators, when the activity is low it may be possible to effect an adaptation of the enzyme by a preliminary injection of small doses of novocain [4, 21]. In Hungary, novocainazoprotein immune sera have been prepared, which, on injection, raise the lethal dose of novocain for rabbits from 60 to 80 mg/kg.

Thus, according to our observations, in endarteritis obliterans patients the novocainesterase blood serum activity is less than normal, and after novocain block it tends to become lowered further still.

In patients of Groups I and V, the excretion of unchanged novocain in the urine after the block continues for more than eight hours. The amount of excreted novocain is very small, and is independent of the concentration of novocain in the body.

In patients with endarteritis obliterans in all forms and phases of the disease, with the exception of sympathectomized patients, the excretion of paraaminobenzoic acid formed by breakdown of novocain is considerably reduced.

SUMMARY

In vitro and in vivo experiments have demonstrated a fall in novocainesterase activating the destruction of novocain in the blood of patients with endarteritis obliterans.

The activity of the enzymatic system catalyzing acetylation of paraaminobenzoic acid – one of the products of novocain metabolism – is as well inhibited.

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THE EFFECT OF STAPHYLOCOCCAL ENTEROTOXIN ON GASTRIC AND INTESTINAL MOTILITY

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In recent years, considerable success has been achieved in the study of staphylococcal enterotoxin. However, the mode of action of the toxin has been comparatively little investigated.

J. Lavergne, J. C. Burdin and J. Sommelet [4] concluded from experiments on two cats that there was no sympathotropic action.

M. Bayliss [3] suggested that the direct action of the toxin on the peripheral nervous system was of considerably greater importance in the etiology of vomiting than was any direct action on the vomiting center.

J. Richmond and C. Reed [5], in their experiments on isolated sections of rabbit intestine, concluded that the staphylococcal enterotoxin exerts a direct action on the smooth muscle of the intestine.

Our observations were made on cats, kittens, and puppies which were given filtered extracts of the toxin intravenously, per os, and intraperitoneally; this caused gastrointestinal disturbance (diarrhea and vomiting) as well as central nervous effects (staggering gait, tremor, convulsions).

These observations were our starting point in the investigation of the mode of action of enterotoxin filtrates. We therefore carried out several experiments on adult cats under urethane anesthesia and with no interference to the nervous connections. Altogether, we investigated 51 cats varying in weight from 2,200 to 4,000 grams.

Filtrates of the enterotoxin were taken from staphylococcal strains which were effective in producing the gastrointestinal syndrome in cats, kittens and puppies.

EXPERIMENTAL METHODS

The method of preparing the enterotoxin filtrate was as follows. A 24-hour agar culture of the staphylococcus, known to produce the enterotoxin, was transplanted to a nutrient medium and incubated in a thermostat for three days. An amount of CO_2 equal to 25-30% of the volume of the vessel was introduced daily. On the fourth day the culture was filtered through a sterile steatite filter. In order to destroy the thermolabile hemolysin, the lethal filtrate of the toxin was heated for 30 minutes in a bath of boiling water. In order to exclude any effect of the nutritive medium, we carried out a control experiment in each group, using uninfected medium.

In the first experiments, we studied the effects of the filtrates on the gastrointestinal motility of the cat in situ.

The experiments were carried out as follows (using the principle of N. P. Nikolaev as modified by the department of Pharmacology of the Lvov Medical Institute). A laparotomy was performed under urethane anesthesia. After we cut the omentum and placed ligatures on the greater curvature of the stomach, small, and large intestines, we introduced a glass cylinder (13 cm long and 7 cm in diameter, open at both ends) into the peritoneal cavity. The cylinder was held in a vertical position in a holder, and 100-150 ml of Ringer-Locke solution heated to 38-39°C was poured through its upper end into the peritoneal cavity. Each of the three ligatures from the stomach, small, and large intestine were brought to the outside and connected to the recording system. The contractions were recorded on ordinary paper by glass ink recorders attached to levers.

After recording the normal contractions of the stomach and intestines, 2 ml per 1 kg of the filtrate were given intravenously, but the amount was not allowed to exceed 6 ml.

EXPERIMENTAL RESULTS

Twenty-one experiments on the effect of staphylococcal enterotoxin filtrates on gastric and intestinal motility were carried out. A record was made of the changes in motility of the stomach and intestine as affected by the staphylococcal enterotoxin filtrate. Injection of the filtrate caused an increase in gastrointestinal activity.

Fig. 1. The effect of staphylococcal enterotoxin filtrate (strain No. 1594) on the motility of stomach and intestine in the cat. Shows increase in contractions and tone.

The effect on both tone and rate of contraction is shown in Fig. 1.

In six control experiments with filtrates from uninfected media and from nontoxic strains of staphylococcus, there was no effect on the motility of the stomach or small intestine. In the experiments on the effect of the medium, there was a reduction in tone of the large intestine, and in two experiments the gastric tone was also reduced.

Having established the effect of the filtrate on the motility of the gastrointestinal tract, we then studied its action on the stomach and intestine after their motility had already been reduced by other means. For this purpose we used adrenalin, atropine, and papaverine.

After the motility of the intestine had been reduced in this way, the enterotoxin filtrate was given intravenously. Altogether, 17 experiments were carried out.

In adrenalin-treatedanimals, intravenous injections of enterotoxin filtrate caused an increase in tone of all parts of the gastrointestinal tract. There was an increase in the motility of the small and large intestine. In animals which had been treated with papaverine, there was a small increase in tone of all parts after enterotoxin filtrate was given. After atropine administration, the effect of enterotoxin filtrate was much less marked. There was sometimes a small effect on the large intestine.

For a more complete investigation, we carried out experiments with enterotoxin filtrates on spinally transected animals. Seven of these experiments were performed.

These gave similar results. However, spinal section caused a considerable change in the rhythm of the contractions, there being a greater change in the actual contraction movement than in the relaxation.

Finally, we studied the effect of "pure" enterotoxin filtrates on isolated sections of cat and rabbit intestine both with and without preliminary treatment with adrenalin and gynergen, or with cholinergic substances such as atropine, pylocarpine, and carbocholine.

These experiments showed that the staphylococcal enterotoxin filtrate increases the motility of isolated gut sections, increasing the tone, amplitude, and frequency of the contraction. Thus, there was an increase in tone in 47 out of 58 experiments, an increase in amplitude in 42, and an increase in frequency in 27 experiments. The control experiments showed that the effect of the sterile medium is to reduce the tone as well as the amplitude and frequency of the contractions.

Filtrates of nontoxic staphylococcal cultures and filtrates of sterile media also reduced the motility of isolated sections of intestine (Figs. 2, 3).

Fourteen experiments were carried out to find the effect of enterotoxin filtrates on isolated sections of intestine which had been previously treated with adrenalin. After recording the normal contractions of a strip of cat intestine, 1-3 drops of 0.1% adrenalin in 100 grams of Tyrode solution were introduced. After 10-15 minutes, 3 ml of enterotoxin filtrate was added.

These experiments showed that the effect of the enterotoxin filtrate on a toneless loop of small intestine was to restore the tone and to increase the frequency and amplitude of the contractions.

In further experiments, adrenalin was added after the enterotoxin. When this was done the adrenalin failed to produce a complete relaxation.

Fig. 2. Reaction of isolated strip of small intestine to staphylococcal enterotoxin filtrate (strain No. 1197), time intervals 20 seconds.

Fig. 3. Abolition of motility after the addition to a strip of small intestine of a non-enterotoxic staphylococcal filtrate (strain V4D-46), experiment No. 156. Time intervals, 20 seconds.

Nineteen experiments were arranged to investigate the action of staphylococcal enterotoxin filtrate on isolated strips of the small intestine of a cat already stimulated by gynergen. These experiments showed that the enterotoxin caused a further increase of tone and frequency of contraction over and above that caused by the gynergen.

In 18 experiments with atropine, it was shown that this drug considerably reduces the stimulant effect of the enterotoxin on isolated strips of small intestine. The addition of the enterotoxin after atropine reduced the tone, amplitude, and frequency of the contractions. In 14 experiments, after stimulating the motility of strips

of rabbit small intestine with the enterotoxin, we added three drops of a 0.1% solution of atropine. The atropine caused a considerable reduction in tone and in the amplitude of the contractions which had been increased by the action of the enterotoxin.

The following 43 experiments were carried out with pilocarpine and carbocholine. After the stimulant action of pilocarpine, the addition of enterotoxin filtrate had no clear effect, and in half of the experiments there was even some reduction in the amplitude of the contractions. Similar effects were noted with carbocholine. Thus in experiments the typical stimulant effect of the enterotoxin filtrate was not shown.

Our experiments showed, therefore, that the staphylococcal enterotoxin filtrate applied to cats in situ had a stimulant effect on gastrointestinal motility. The effect was shown most strongly in the large intestine, less strongly in the small intestine, and least of all in the stomach.

It was established that there was an increase in the tone of the smooth muscle of the gut in animals previously treated with adrenalin and papaverine. Some reduction in motility was found in animals treated with atropine. The increase in smooth muscle tone caused by the enterotoxin in adrenalin-treated animals shows that the adrenergic nerve fibres of the intestine play hardly any part in this action.

The absence of any increase in smooth muscle tone in animals receiving the enterotoxin after preliminary treatment with atropine, shows that the enterotoxin can stimulate the cholinergic nerves. The same conclusion applies in the case of the animals treated with papaverine.

The increase in the motility of stomach and intestine resulting from the enterotoxin applied to animals after spinal section, confirms the cholinergic nature of the action. Experiments with isolated strips of intestine show that the enterotoxin directly increases the motility. This effect is less clearly shown with atropine, and is reduced by subsequent application of the latter. Sympathotropic substances do not change the above reaction.

The effect is progressively less in the large intestine, small intestine, and stomach, respectively.

Thus sympathomimetic substances have no effect on the sensitivity of the animal to the staphylococcal enterotoxin.

The effect of the enterotoxin is mediated by cholinergic nerves, and is shown by an increase in the muscular tone of the intestine and by a reduction in this reaction in atropinized animals, as well as by a reduction in tone when atropine is given after the enterotoxin.

SUMMARY

It was established in experiments on cats in situ that enterotoxin excites the motor activity of organs of the gastrointestinal tract. The most pronounced reaction to staphylococcus enterotoxin occurred in the large intestine, then the small intestine and stomach respectively. Pharmacological substances which enhanced the sympathetic reactions had no effect on the sensitivity of the animal to staphylococcus enterotoxin. Experiments were carried out to study the effect of enterotoxic staphylococci on the motor activity of the isolated strips of small intestines of cats and rabbits. It was shown that enterotoxin excited the motor activity and increased the tonus, amplitude and frequency of contractions. On the basis of these experiments it may be suggested that staphylococcus enterotoxin possesses a cholinergic effect.

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BIOCHEMISTRY AND BIOPHYSICS

THE ROLE OF THE NERVOUS SYSTEM IN THE REGULATION OF TISSUE RIBOFLAVIN CONTENT

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It is well established that animals having a diet inadequate in riboflavin develop a serious illness, ariboflavinosis, which is characterized along with other symptoms by the development of trophic disturbances and retardation of regenerative processes.

The trophic ulcers that develop in the course of ariboflavinosis resemble in some respects those trophic ulcers so resistant to therapy that arise after the intactness of individual nerves has been disturbed. This circumstance led us to the thought that there may be an association between riboflavin levels in the organism and the trophic function of the nervous system.

To clarify this question three series of experiments were performed in the course of which we studied the role played by the nervous system in the accumulation of riboflavin in the tissues of the animal organism.

EXPERIMENTAL METHODS

The experiments were done on white rats weighing 150-200 g. The animals were maintained on a full synthetic diet with supplementation of the essential vitamins.

In choosing the organ to be denervated we decided on the liver as being richest in riboflavin and therefore the most suitable.

Our first series of experiments was performed on 45 white rats with denervated livers.

Following the operation within 2, 4, 10, 20, 30, 60 and 70 days the animals were sacrificed in groups of 5 to 8 and the riboflavin content of their organs (liver, kidneys, heart, striated muscles) was determined.

Tissue riboflavin determinations were made by the S.E. Kaplan method.

This procedure is based on extraction of riboflavin from the organs by means of methyl alcohol and determination of the degree of fluorescence under ultraviolet rays.

EXPERIMENTAL RESULTS

Fig. 1 presents the riboflavin content of various organs of rats that had their livers denervated and the tissues removed for study at the different times after the operation.

As can be seen from the figure, the amount of riboflavin present drops sharply as soon as the 2nd day following liver denervation this taking place within liver tissue itself (by 49%) as well as in other organs (in kidneys by 37%, in heart by 50%).

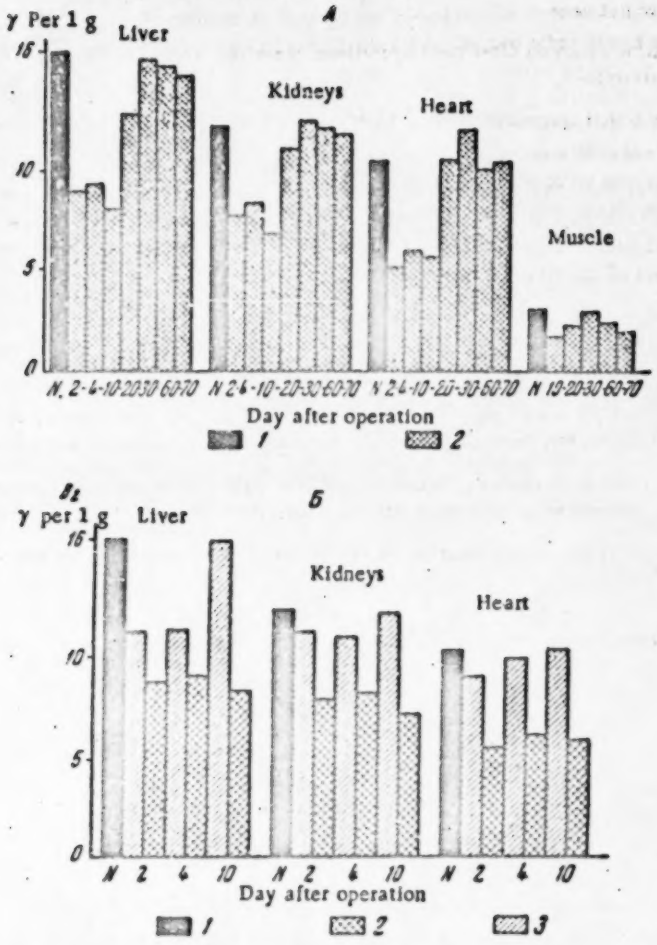

Fig. 1. Riboflavin content of rat organs at various time intervals after the operation denervating their livers.
1) normal; 2) after denervation; 3) control trauma. A, B) different experimental series.

The riboflavin level continued depressed even on the 4th and 10th days after the operation. By the 20th day the riboflavin would rise in the liver as well as the other organs reaching normal levels only by the 30th postoperative day.

It may be supposed that by that time there developed a certain degree of compensation for the disturbed nervous connections although the mechanism of such an adjustment remains unexplained.

It must be pointed out that the liver denervation performed by us was incomplete as we did not disturb the fibers accompanying the blood vessel net.

After we had performed the experiments with the denervated livers, we felt a need to determine whether the alterations in the tissue riboflavin levels were due to nerve fiber destruction only or whether they were a post-traumatic effect.

To clarify the point a second experimental series was run.

Twenty-five animals of this group were maintained under conditions similar to those in the first group

and were then subjected to procedures undergone by the animals of the first series with the sole exception that their livers were not denervated.

At various time intervals after their operations, these rats were sacrificed and the riboflavin content of their organs then determined.

The influence that traumatization of the liver has upon riboflavin content in the organs of white rats is shown in Fig.1, 3.

As can be seen from the Figure, operational trauma leads to some depression of the riboflavin content of the liver but this is much less than when accompanied by denervation. The amount of riboflavin within the liver in these experiments retumed to normal as soon as the 10th day, i.e., much more rapidly than after denervation. The level of riboflavin in the other organs remained almost unaffected.

It follows, therefore, that the riboflavin depression obtained in the first series of experiments occurs in the organs and tissues of the rats as a result of liver denervation and that the nervous system of the liver, when intact, must participate in some basic manner in. the riboflavin metabolism of the organism.

In order to clarify the role played by the liver in the riboflavin alterations within the organs and tissues which occur after the liver had been denervated, a third series of experiments was performed.

In twenty-five animals of this group, 0.5 cm of the right sciatic nerve was resected. At various times after this operation, the rats were sacrificed and the riboflavin content of their organs was determined.

The quantity of riboflavin present in the rat tissues after the sciatic nerve had been sectioned is represented in the table.

Riboflavin Present in Rat Organs After Resection (0.5 cm) of the Right Sciatic Nerve (in μg)

Rivoflavin content in μg	Normal rats				After operation											
					4th day						10th day		69th day		75th day	
								Calf muscle								
	Liver	Kidney	Heart	Calf muscle	Liver	Kidneys	Heart	Right	Left	Right	Left	Right	Left	Right	Left
Minimum	13.6	10.4	8.4	1.8	14.8	12.4	10.0	0.2	2.8	0	2.2	0.2	2.2	0	2.8
Maximum	19.6	12.5	12.1	3.4	16.4	14.6	10.4	0.3	2.4	0.4	3.4	0.4	3.6	0.4	3.8
Average	16.0	12.3	10.4	2.3	15.5	13.2	10.1	0.25	2.6	0.2	2 8	0.3	2 6	0.2	2.8

As is evident from this table, the amount of riboflavin present in the liver, kidneys and the heart was the same after resection of the sciatic nerve as before the operation. Only in the right calf muscle was there a sharp diminution in the riboflavin content after the right sciatic nerve had been resected, this decrease never becoming compensated.

The amount of riboflavin present in the left calf muscle (with undisturbed innervation) was 2.2-3.8 μg per 1 g, this corresponding to the riboflavin present in the muscles of normal rats.

This evidence indicates that irreversible destruction of a nerve leading to a muscle causing the nerve to degenerate provokes a constant diminution in the amount of riboflavin in the calf muscle while in the other muscles and organs such alterations do not take place.

In this manner there was established the basic difference between disturbing the intact innervation of the liver and sectioning the sciatic nerve in so far as these maneuvers affect the riboflavin content of the tissues. Severing the sciatic nerve depressed only the quantity of riboflavin present in the denervated muscle tissue, not affecting the content of this vitamin in other organs and tissues, while denervation of the liver altered the amount of riboflavin present not only in the liver but also in other organs.

This leads to the conclusion that the nervous system participates not only directly in the regulation of the riboflavin contents of tissues but also indirectly through its effect on the liver which, when denervated in our experiments, resulted in prolonged depression of the riboflavin content in other organs and tissues.

SUMMARY

The nervous system has two ways in which it affects the riboflavin level within the organism. It does so directly as in the experiment in which severing the portion of the sciatic nerve leading to a calf muscle depressed the riboflavin content of that muscle almost to the vanishing point. It does so indirectly as in the experiment in which denervation of the liver decreased the riboflavin content in kidneys, heart and striated muscles.

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ALTERATIONS IN FETAL RABBIT PROTHROMBIN TIME AT DIFFERENT STAGES OF GESTATION AND DURING ASPHYXIA

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It is well known that, in comparison with the adult, the newborn has a marked prolongation of its prothrombin time [1, 2, 3, 8].

In the opinion of many authors this is the result of exogenous and endogenous vitamin K deficiency [4, 5, 6, 8, 10].

However, our preceding observations have demonstrated that the prothrombin time in the newborn begins to diminish beginning with the 3rd to 5th day of life even when these infants receive no vitamin K.

In addition, it has been shown that the liver of infants dying as a result of intracranial hemorrhages had adequate amounts of vitamin K even when the prothrombin time during life had been shown as being prolonged. Evidently, the prolongation of the prothrombin time in the newborn must be associated not only with K hypovitaminosis but also with many other factors.

The present study had as its goal the observation of prothrombin time changes in the period of intrauterine ontogenesis and the alterations which occur when the fetus enters into extrauterine life, i.e., starts the period of pulmonary respiration and, also, how prothrombin time is affected in the fetus by asphyxia.

EXPERIMENTAL METHODS

The experiments were conducted upon intratubal rabbit fetuses. The pregnant rabbit received an injection subcutaneously of a 5% solution of hexanastab, the dose being 4 cc per 1 kg of animal weight. After the rabbit had gone to sleep, it was placed into a bath of physiological saline the thermostatic control being set at a temperature of 37.5-38°, the abdominal cavity was opened, the uterine horns brought out and the fetuses removed in turn. The blood for determining the prothrombin times was taken from the fetal hearts. The fetal prothrombin time determinations were made by the Kazantseva modification of the Quick method [2, 3].

EXPERIMENTAL RESULTS

Prothrombin time in the period of intratubal ontogenesis. In 16 fetuses aged 20 to 24 days it was not possible to determine the prothrombin time by the Quick-Kazantseva method as the blood did not coagulate even after two hours.

In fetuses at the stage of 26-28 days of intratubal life (35 experiments) the prothrombin time fluctuated between 70 to 20 seconds, averaging 40.4 ± 0.6 seconds. Shortly before birth, i.e., between 29th and 31st days of intrauterine life (40 experiments), the prothrombin time varied from 35 to 15 seconds averaging 26.4 ± 0.8 seconds. In this last group there was observed a definite difference in fetuses at the 29th day of intratubal life and

fetuses at the 31st day of intratubal life. The 29 day fetuses averaged a prothrombin time of 25 seconds while the 31 days fetuses averaged 18-20 seconds.

TABLE 1

Prothrombin Time in Fetuses Between 26-31 Days of Intratubal Life as They Entered Stage of Pulmonary Respiration

No. of exp.	Fetal age (days)	No. of fetuses	Prothrombin time (in seconds)		
			Preg. rabbit	Fetuses (untied umbil. cords)	Fetuses (tied umbil. cords)
1	26	4	13	40	35
				35	35
2	27	8	12	45	46
				48	48
				45	40
				46	48
3	27	6	15	55	60
				55	55
				55	60
4	27	8	15	45	45
				48	43
				45	45
				45	40
5	28	8	16	55	55
				60	57
				60	60
				50	53
6	29	4	13	35	30
				33	35
7	29	8	12	35	30
				35	33
				30	35
				35	35
8	30	8	14	25	22
				25	25
				23	25
				24	20
9	31	8	12	18	20
				15	15
				20	18
				18	20
10	31	8	14	20	18
				18	20
				18	18
				20	20
	Average . . .			35.4±0.7	34.9±2.3

TABLE 2

Prothrombin Time in Fetuses at 26-28 Day of Intratubal Development During Asphyxia

No. of exp.	Fetal age (days)	No. of fetuses	Prothrombin time (in seconds)		
			in preg. rabbit	in control fetus	in asphyx. fetus
1	26	6	14	25	20
				25	23
				25	20
2	26	6	18	30	40
				32	40
				30	40
3	27	8	12	40	45
				38	48
				40	45
				42	46
4	28	6	12	27	32
				25	33
				25	35
5	28	8	15	25	35
				25	36
				23	40
				23	32
Average				29.4±2.02	35.8±1.1

As the fetus becomes older, along with diminution of the prothrombin time, there is also observed a change in the character of the coagulation reaction: in 24-day fetuses the blood does not clot and after 2-4 hours of standing there appear in it only a few scarcely visible grains of fibrin; in the blood of 26-28 day fetuses there was noted the appearance of fibrin clumps settling on the walls of the tube; in the blood of fetuses in the last stages of gestation there was observed the development of heavy thickenings and clumps, while just before term (31st day) a definite clot floating freely in the tube was present. In the female pregnant rabbit, as the blood coagulated, a firm clot filling the entire lumen of the tube and tightly adhering to its walls was formed.

Alterations in prothrombin time in fetuses during the period of transition from intratubal to extratubal life. In this experimental series 35 animals were studied. After tying the cord the fetus was immediately removed from the mother and placed on a cotton pillow; within several seconds there would begin respiration and movement. Within 10-20 minutes after respiration had begun, blood was taken from the heart to determine the prothrombin time. As controls, the fetuses in the other horn of the mother (that had not been separated from the placenta) were used. The results obtained are shown in Table 1.

As can be seen from this table, there were no significant prothrombin alterations as the fetuses entered into the phase of pulmonary respiration.

Prothrombin time alterations produced by asphyxia. These studies were conducted on fetuses between 26 to 28 days of gestation (17 fetuses) and 30-31st day (30 fetuses).

Twenty minutes after cord ligation blood was drawn from the fetus immersed in the physiological saline solution and the prothrombin time determined. As a control, the fetus in the other uterine horn which had not had its cord tied was used.

TABLE 3

Prothrombin Time in Fetuses at 29-31 Day of Gestation During Asphyxia

No. of exp.	Fetal age (days)	No. of fetuses	Prothrombin time (in seconds)		
			In preg. rabbit	In control fetus	In asphyx. fetus
1	29	8	17	35 35 35 35	45 45 45 45
2	29	8	15	30 25 25 25	35 28 30 30
3	30	6	10	20 20 20	30 30 30
4	30	8	12	20 22 22 22	25 26 29 27
5	30	6	10	23 23 24	28 28 28
6	30	8	12	20 20 20 20	25 25 25 25
7	30	8	13	20 23 20 25	28 30 25 28
8	30	8	14	25 28 28 30	30 35 30 35
Average				24.6±0.9	30.9±1.1

Tables 2 and 3 demonstrate that prothrombin time lengthens in the fetuses as asphyxia develops. Thus the control fetuses in 26-28 days of gestation had an average prothrombin time of 29.4 seconds, while fetuses of same age that were in a state of asphyxia had an average prothrombin time of 35.8 seconds.

Control fetuses in 29-31 day of gestation had a prothrombin time of 24.6 seconds while in fetuses being in an asphyxial state the time averaged 30.9 seconds.

DISCUSSION OF EXPERIMENTS

The above experiments demonstrate that during intratubal ontogenesis variations in prothrombin time occur; as the fetus matures, the prothrombin time diminishes gradually. This permits us to believe that prolongation of prothrombin time which is observed in the newborn is not associated with the transition to pulmonary respiration but is a continuation of changes which had begun during gestation. To understand the mechanism involved, one must take into account the changes undergone by the blood in the period of intratubal development. A primary consideration is the blood content of fibrinogen the amount of which increases as the embryo grows [9].

The low levels of prothrombin and fibrinogen during gestation point to the liver as not having had time to develop its synthetic function. At birth the increase in blood prothrombin and fibrinogen levels is not terminated but continues. Apparently, the comparative (with adult) decrease in prothrombin which is observed in the newborn is a consequence of an inadequately developed prothrombin capacity of the liver, i.e., the prolongation of the prothrombin time seen in the newborn is a reflection of one of the physiological characteristics of the organism at that stage of life.

SUMMARY

Experiments were conducted which demonstrated that prothrombin time diminishes gradually during gestation of the fetuses. The suggestion is made that this may be associated with the appearance of the prothrombin formation function of the liver. This function is not fully developed at birth but continues maturing. This and not vitamin K deficiency is suggested as the reason

for the comparatively prolonged prothrombin time seen during the first few days of independent life. Prothrombin time is greatly increased by asphyxia.

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*In Russian.

PHARMACOLOGY

THE EFFECT OF BODY TEMPERATURE ON TRANSMISSION OF EXCITATION IN THE SUPERIOR CERVICAL GANGLION AND THE ACTION OF GANGLION-BLOCKING PREPARATIONS

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Artificial lowering of body temperature has recently attracted much attention from surgeons who have been using hypothermia in certain operative procedures. Various ganglion-blocking agents are used in conjunction with hypothermia. It is postulated that these measures lower the metabolism and the general reactivity of the organism [8]. It is therefore important to investigate autonomic ganglionic transmission of excitation and the action of substances which depress this transmission under conditions of general cooling.

EXPERIMENTAL METHODS

Experiments were performed on cats under urethane anesthesia. The effect of hypothermia on transmission of excitation in the superior cervical sympathetic ganglion was studied. The preganglionic sympathetic trunk was stimulated by square-pulse stimuli from an electronic stimulator; the duration of the stimuli was 0.1-0.2 m sec with constant amplitude and variable frequency for each experiment. Contraction of the nictitating membrane was recorded on a smoked drum kymograph. Increasing the frequency of stimuli led to partial relaxation of the nictitating membrane, which corresponded to the least reaction. Return to the initial, optimal, frequency of stimulation restored the mechanogram to its former magnitude. Using the oscillographic method of recording, the ganglion action potentials were led off from the postganglionic fibers through a single channel balanced A. C. amplifier and recorded by means of an oscillograph. The biopotentials were photographed during stimulation at 2-3 second intervals. The state of ganglionic transmission of excitation was judged by the maximal rhythm reproducible without transformation and by the time during which a decrease of biopotential amplitude took place. The animals were cooled in a double-walled metal bath filled with water at 3-4°. The rectal temperature was measured with a mercury thermometer. In some of the experiments cats decerebrated at the collicular level were used. No perceptible difference could be demonstrated between urethane-anesthetized and decerebrate animals, which corresponds to the data of M. G. Larrabee and I. M. Posternak [9], who showed that urethane in narcotic doses did not affect transmission of excitation in sympathetic ganglia.

RESULTS

Lowering the body temperature to 26-25° did not exert any appreciable effect on contraction of the nictitating membrane. The magnitude of the mechanogram usually remained unchanged. The least reaction often appeared during the initial period of cooling (35-29°) at higher frequencies of rhythmic stimulation than was the case under normal conditions. Further lowering of body temperature produced diminution of lability which was expressed in a shift of the least reaction towards lower frequencies. When the body temperature dropped below 23-20°, however, the lability and magnitude of nictitating membrane contraction were markedly lowered. If

cooling was continued it was possible to observe complete absence of contractions regardless of the frequency of stimulation. In some experiments the lowering of lability and amplitude of the nictitating membrane contractions occurred as early as 27-36°. Warming of the animal led to restoration of the mechanogram value.

Oscillographic records showed that the amplitude of the action potentials at low rates of rhythmic stimulation (5-10 cps) did not change substantially with the onset of hypothermia. At the same time it was possible to observe a progressive diminution in the capacity of the ganglion to reproduce without transformation the higher rhythms of stimulation (20-50 cps; Fig. 1). Diminution of lability was expressed by the more rapid decrease in the amplitude of the electric potentials than in the normal. When the body temperature reached 27-25° the frequency of the biopotentials recorded from the ganglion did not exceed 10-15 cps; the latent period of biopotentials synchronous with the stimulation became markedly longer.

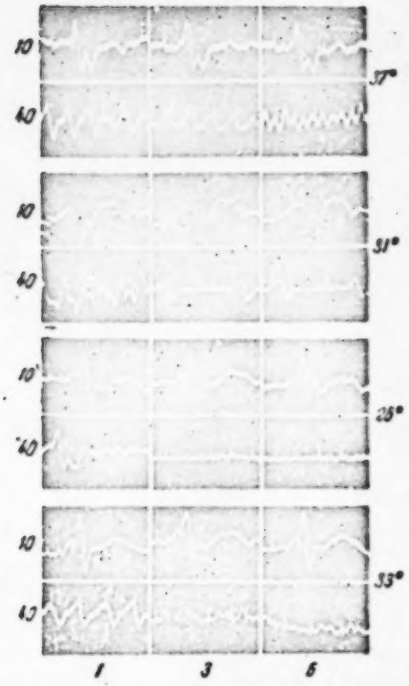

Fig. 1. The effect of body temperature on postganglionic fiber potentials. Figures at left (in this and other figures) refer to frequencies of stimulation of the preganglionic trunk (in cps).*

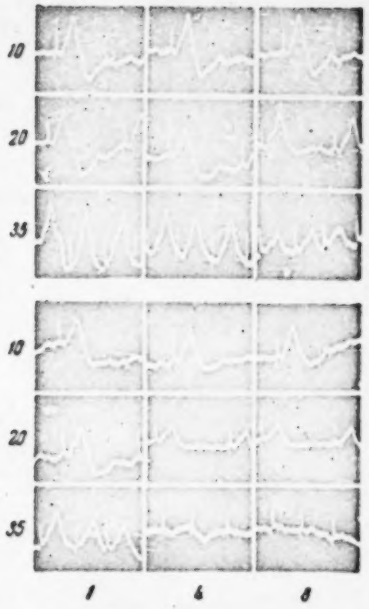

Fig. 2. The effect of body temperature (36°) on the ganglion-blocking action of hexonium. Arrow marks administration of hexonium.*

Oscillographic records failed to show a phase of increased lability as seen in the experiments with recording of the nictitating membrane contractions. These differences may be explained by the fact that lowering of temperature evokes an increase of tonus in smooth muscle [11], which tends to prevent its relaxation despite the development of the least reaction in the ganglion cells.

It must be noted that cooling to 33-29° often produced considerable increase of the slow negative component of the bioelectric potentials recorded from the postganglionic fibers. These changes were particularly apparent when the frequency of stimulation did not exceed 10-15 cps, under those conditions when the true form of the biopotentials could still be observed [2].

The data obtained correspond with those available in the literature. Thus, D. W. Bronk and R. Pamphrey [3] established that on lowering the temperature progressive lowering of synchronous electric activity in the stellate ganglion takes place. W. W. Douglas and J. M. Ritchie [5] did not observe any change of excitation in the superior cervical ganglion of rabbit at low rates of stimulation carried out with cooling.

* Transliterated from Russian – probably hexamethonium.

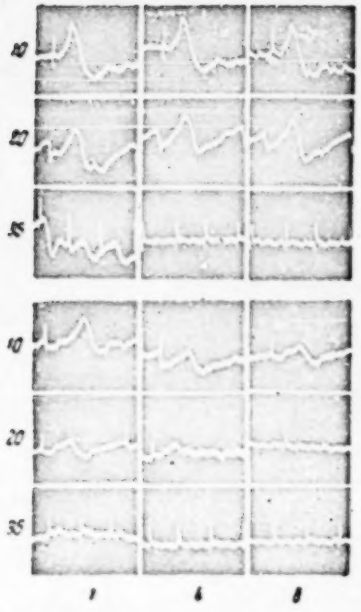

Fig. 3. The effect of body temperature (25°) on ganglion-blocking action of hexonium. Arrow marks administration of hexonium.

Studies on the effect of hypothermia on the action of ganglion-blocking agents included investigation of sparteine sulphate, tetraethylammonium iodide and hexonium. As was shown by Kharkevich [1], sparteine and tetraethylammonium hamper transmission of excitation from the ganglion first of all at high frequencies of stimulation. Analogous results were obtained in the present work with hexonium.

The blocking effect of the substances being studied increased as the body temperature was lowered. This was expressed in the mechanograms by lowering of the height of contraction in cooled animals by doses of ganglion-blocking agents which, under normal conditions of body temperature, caused only a drop in lability without any effect on the size of the mechanogram at optimal frequency of stimulation. However, relaxation of the nictitating membrane corresponding to the least reaction was often less pronounced at low temperature.

Oscillographic records showed that the decrease in amplitude of the biopotentials following administration of hexonium, sparteine and tetraethylammonium was more pronounced the lower the body temperature (Figs. 2 and 3). It should be stressed that the increase in blocking action only became apparent when the body temperature was lowered to 27-26°. Complete abolition of excitatory transmission at 24°, as shown by the oscillographic records, required doses of hexonium, sparteine and tetraethylammonium which were $1\frac{1}{2}$-2 times smaller than those at 36-37°. Analogous data were obtained by Taddei and Mosetti [12] using arfonad, a ganglion-blocking agent. Restoration of excitation transmission in cooled animals occurred 2-3 times more slowly than at normal body temperature. Therefore repeated administration of the substances led to greater disturbances than at normal temperature.

There are references in the literature to the dependence on temperature of acetylcholine production and of the sensitivity to it of choline-reactive structures. However, Kostial and Vouk [7] found, in their experiments on the isolated rat cervical ganglion, that the amount of acetylcholine on stimulation of the preganglionic trunk did not alter appreciably at temperatures within the range of 40 to 20°. Pascoe [10], using the same experimental preparation, did not find any changes of the depolarizing action of acetylcholine on altering the temperature from 38 to 3°.

In the present experiments with intravenous administration of acetylcholine no substantial differences in nictitating membrane contractions could be detected when comparing normal and cooled animals. It can therefore be suggested that the diminution of lability evoked by hypothermia does not depend on impairment of the synthesis and destruction of acetylcholine. As shown by biochemical studies, the consumption of glucose and oxygen by the sympathetic ganglion drops sharply with lowering of temperature [4, 6]. Evidently, it is the decreased intensity of metabolic processes which leads to lowering of the functional activity of ganglion cells as expressed by the shift of the least reaction towards lower frequencies.

SUMMARY

Decrease in lability in the upper cervical ganglion which was manifested by the shift of pessimum towards low frequencies was observed in cats when their body temperature was gradually decreased from 37°C to 25-24° C. The response to rare (optimal) rhythms of stimulation was almost unchanged. The effect of the substances, which block the transmission of excitation in the ganglion was almost unchanged in cooling from 37° C to 33-31° C, but with lower temperatures of the body their effect was enhanced. If the body temperature is brought back to normal the functions of the ganglion were restored.

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CORRELATION OF CHEMICAL STRUCTURE AND PHARMACOLOGIC ACTION OF SOME NEW ANTICHOLINE AGENTS*

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Studies on the correlation of chemical structure and pharmacologic action were carried out on four groups of anticholine agents: 1) the pentaphen or diethylaminoethyl ester of phenylcyclopentane carboxylic acid group [9, 10, 1, 4, 3], 2) the diphazine or diethylaminoacetylphenothiazine group [8, 4, 3], 3) the alphamethyldiphazine group containing a methyl group in the alpha position with respect to the nitrogen of the aminoacetyl part of the molecule [7], 4) the arpenal or diethylaminopropylamide of diphenylacetic acid [10, 3].

Each group was composed of three preparations: the hydrochloride containing a tertiary nitrogen atom, and two alkyl iodides (methyl iodide and ethyl iodide) containing a quaternary nitrogen atom. In the pentaphen and diphazine groups there were also the methylsulfomethylates. The structure of all these compounds is shown in the table (see below). The majority of the substances were synthesized by N. B. Khromov-Borisov and A. M. Yanovitskaya (I LMI), the arpenal group was synthesized in Professor A. L. Mndzhoyan's Laboratory (Academy of Sciences of the Armenian SSR), while pentaphen hydrochloride was obtained from VNIKhVI.

EXPERIMENTAL METHODS

Experiments were performed on cats under urethane and hexenal anesthesia and on decerebrate animals. The vagosympathetic trunks were dissected in the animal's neck; the sympathetic nerve was isolated on the left, and the vagus on the right. Ligatures were applied to the nerves, the nerves were cut and introduced into cannula-like electrodes. During the experiment the preganglionic sympathetic nerve trunk was subjected to prolonged stimulation with square-wave pulses from an electronic stimulator at a frequency of 16 per second (duration of each pulse 0.1 millisecond). The preparations were injected intravenously. The effect of the substances on transmission in the superior cervical sympathetic ganglion was determined by their ability to lower the height of contraction of the nictitating membrane [6] (Fig. 1). In control experiments it was established that the preparations in similar doses did not affect contractions of the nictitating membrane elicited by stimulation of postganglionic fibers (Fig. 2) and adrenalin.

The peripheral segment of the vagus was subjected for 5 seconds with an interval of 3-5 minutes to periodic stimulation with induction current with a frequency of 35 impulses per 1 second. The effect of the compounds on conduction in the vagus was determined by their ability to abolish the fall of blood pressure caused by stimulation of this nerve (Fig. 1).

The effect of the preparations on the muscarine-sensitive choline-reactive systems was evaluated by their ability to abolish the fall of blood pressure occurring in response to intravenous administration of acetylcholine (Fig. 3).

The blocking effect of the preparations on the nicotine-sensitive choline-reactive systems of the adrenals and the carotid sinus zones was determined by their ability to abolish respiratory stimulation and rise of blood pressure following intravenous administration of nicotine (0.02-0.03 mg/kg) or of dicholine ester of suberic acid

* Communicated at the 603rd meeting of the I.M. Sechenov Society, Leningrad on April 19, 1955.

(0.01 mg/kg) (Fig. 3). The pressor effect of these preparations is determined chiefly by the action on adrenal medulla, while the respiratory stimulating effect-by the action on the chemoreceptors of the carotid sinus zones [5].

RESULTS

The results of the experiments are presented in the table. All the preparations being studied revealed anticholine action. In each group of substances the alkyl iodides containing a quaternary nitrogen atom showed much stronger anti-acetylcholine action than the corresponding hydrochlorides containing a tertiary nitrogen atom. In this respect the present data agree with the results obtained in studies on other anticholine preparations [7,11,13, 14]. Thus, as regards the ability to abolish the muscarine-like effect of acetylcholine, the alkyl iodides exert a 2-3 times stronger effect than the corresponding hydrochlorides. This feature is still more clearly shown by the determination of the ability of the preparations to prevent the depressor action of the vagus. Pentaphen and diphazine hydrochlorides block vagal conduction in doses of 1.0-2.0 mg/kg, arpenal and alphamethyldiphazine hydrochlorides – in doses of 0.3-0.5 mg/kg. All the alkyl iodides block vagal conduction when used in doses of 0.03-0.05 mg/kg, exceeding in strength of their action that of the corresponding hydrochlorides by 20-40 times for the penthaphen and diphazine groups, and by 6-10 times for the arpenal and alphamethyldiphazine groups. It was thus found that all the preparations being studied prevented the depressor effect of stimulation of the vagus in doses tens of times smaller than those required to abolish the depressor effect of acetylcholine on intravenous injection. The difference in the doses which block the depressor effects of the vagus and acetylcholine may also depend on the fact that on stimulation of the vagus this effect arises as the result of action on the heart only, while the depressor effect of intravenous acetylcholine is determined predominantly by action on the vessels. Consequently, the difference in the doses of the preparations which block these effects may depend on different sensitivity of the "M"-choline reactive systems of the heart and the vessels. However, administration of a purely anti-muscarine agent – adrenalin – is not associated with such a difference, the two doses being almost identical. This suggests that the preparations being studied may block vagal conduction at the level of the intramural ganglia of the end-organ. Nevertheless, it is impossible to dismiss the hypothesis that, unlike atropine, these substances may also differ in their ability to block muscarine-sensitive receptors of the heart and vessels.

Fig. 1. The influence of diphazine methyl iodide on the depressor effects of vagal stimulation and on contraction of the nictitating membrane elicited by prolonged stimulation of preganglionic sympathetic fibers. Cat, 2.7 kg. Hexenal 150 mg/kg subcutaneously. Records from above down: blood pressure in common carotid artery, contractions of the nictitating membrane, marker for stimulation of sympathetic nerve, marker for stimulation of the peripheral segment of the vagus, time marker (1 minute). Arrows indicate intravenous injections of diphazine methyl iodide in doses of 0.5 and 10 mg/kg.

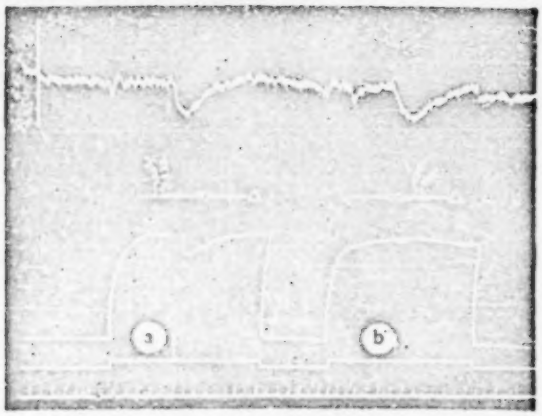


Fig. 2. The influence of pentaphen methyl iodide on contractions of the nictitating membrane elicited by stimulation of pre- and postganglionic sympathetic fibers.
Cat, 3.2 kg. Urethane 1 g/kg intraperitoneally. Records from above down: blood pressure, contractions of the nictitating membrane, marker for stimulation of preganglionic (a) and postganglionic (b) sympathetic fibers, time marker (1 minute). Arrows indicate intravenous injections of pentaphen methyl iodide in doses of 0.3 mg/kg.

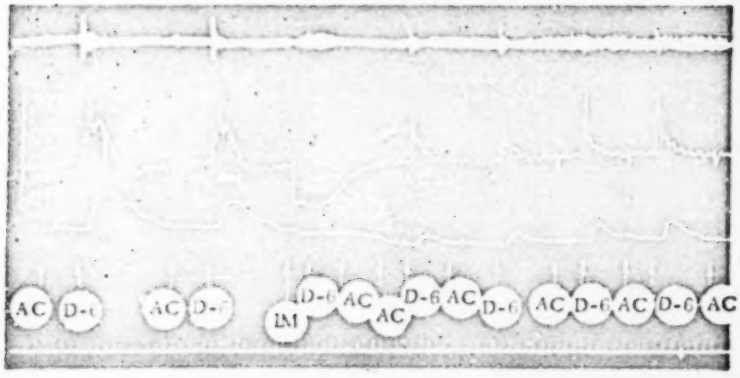


Fig. 3. The influence of penthaphen iodomethylate on the effects of acetylcholine and of dicholine ester of suberic acid.
Cat, 3 kg. Urethane 1 g/kg intraperitoneally. Records from above down: respiration, blood pressure, contractions of the nictitating membrane, time marker (1 minute). Arrows indicate intravenous injections: AC – acetylcholine $1 \cdot 10^{-6}$ in dose of 1 ml, D-6 dicholine ester of suberic acid in dose of 0.01 mg/kg, IM – pentaphen methyl iodide in dose of 3 mg/kg.

Larger doses of the preparations are required for diminution of transmission in the superior cervical sympathetic ganglion, but the relative strengths of action between the substances with tertiary nitrogen atoms and with quaternary nitrogen atoms in their molecules are preserved. Pentaphen and arpenal hydrochlorides exert this action in doses of 2.0-3.0 mg/kg, alpha-methylated diphazine hydrochloride – in doses of 5.0-7.0 mg/kg, and

Minimal Doses of Anticholine Substances (in mg/kg intravenously) and Their Action

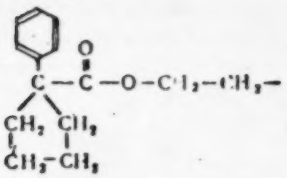
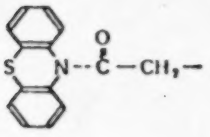
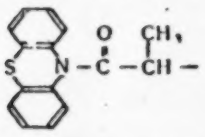
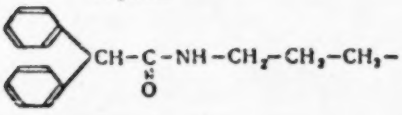
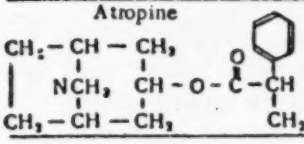
Chemical structure of the preparation		Molecular weight
R I	R II	
Pentaphen 	$-N(C_2H_5)_2(C_2H_5)\cdot HCl$	320.8
	$-N^{+}(C_2H_5)(CH_3)(C_2H_5)$	426.3
	$SO_4CH_3\ -N^{+}(C_2H_5)(CH_3)(C_2H_5)$	415.0
	$-N^{+}(C_2H_5)(C_2H_5)(C_2H_5)$	440.3
Diphazine 	$-N(C_2H_5)_2\cdot HCl$	341.8
	$-N^{+}(C_2H_5)(CH_3)(C_2H_5)$	446.3
	$SO_4CH_3\ -N^{+}(C_2H_5)(CH_3)(C_2H_5)$	438.0
	$-N^{+}(C_2H_5)(C_2H_5)(C_2H_5)$	460.3
Alphamethyl diphazine 	$-N(C_2H_5)_2\ HCl$	362.9
	$-N^{+}(C_2H_5)(CH_3)(C_2H_5)$	468.4
	$-N^{+}(C_2H_5)(C_2H_5)(C_2H_5)$	474.4
Arpenal 	$-N(C_2H_5)_2\cdot HCl$	360.9
	$-N^{+}(C_2H_5)(CH_3)(C_2H_5)$	446.4
	$-N^{+}(C_2H_5)(C_2H_5)(C_2H_5)$	480.4
Atropine  H_2SO_4	—	339.4
Tetraethyl-ammonium CH_3-CH_2-	$-N^{+}(C_2H_5)_3\ Br^{-}$	173.9

Table (continued)

Abolition of depressor effects on		Lowering of the hgt. of contrac. of nictit. memb. kept by prolonged stim. pregang. simpath. fibers	Abolition of Effects Elicited by Intrav. Injec. of Dicholine Ester of Suberic Acid		
intravenous injection of acetylcholine	stimulation of the vagus		Pressor effect	Respiratory stim.	Contrac. nictit. membrane
5.0	0.5—1.0	2.5—3.5	10.0	5.0	5.0
2.0	0.03	0.2—0.3	3.0	3.0	3.0
—	0.03	0.2—0.3	—	—	—
3.0	0.2	0.2—0.3	3.0	3.0	3.0
5.0	1.5—2.0	20.0	10.0	10.0	10.0
3.0	0.05	1.0—1.5	5.0	5.0	5.0
—	0.05	1.0—1.5	—	—	—
3.0	0.05	1.0—2.0	3.0	3.0	3.0
5.0	0.2—0.5	7.0—8.0	7.0—8.0	7.0—8.0	7.0—8.0
1.0	0.03	0.2—0.5	2.0	2.0	—
2.0	0.05	2.0—3.0	3.0—4.0	3.0—4.0	—
6.0	0.3	2.0	5.0	5.0	5.0
2.0	0.05	0.2	2.0	2.0	2.0
2.0	0.05	0.1	2.0	2.0	2.0
0.02	0.01	—	—	—	—
10.0	1.5	0.1	10.0	10.0	10.0

diphazine hydrochloride only in the toxic dose of 20 mg/kg. The alkyl iodide preparations in all the groups block sympathetic ganglionic transmission in doses 5-10 times smaller than the corresponding hydrochlorides (see table).

Abolition of pressor effects, respiratory stimulation and contractions of the nictitating membrane elicited by the dicholine ester of suberic acid was achieved by administration of large doses of the preparations, although in this case, too, the blocking activity proved to be more pronounced in compounds containing a quaternary nitrogen atom in their molecules.

The radical at the quaternary nitrogen atom is also of substantial importance. Among the alkyl iodides the iodomethylates showed stronger anticholine action in most cases as compared with iodoethylates.

The comparison of iodomethylates (methyl iodides) of pentaphen and diphazine with the corresponding methylsulfomethylates failed to reveal any difference in the action of these preparations. At the same time the the solubility of the latter is much higher than the solubility of methyl iodides.

Thus, the main consistent feature traced in all four groups of substances being studied is the manifold increase of peripheral anticholine effects on transition from hydrochlorides to alkyl iodides. In connection with this it is interesting to note that the central anticholine action of the same preparations (anticonvulsant action, ability to influence higher nervous activity) on the contrary either diminishes markedly or disappears completely on transition from hydrochlorides to alkyl iodides, and methyl iodides prove to be the least active [4, 3].

Another feature has been noted: introduction of a methyl group into the alpha position with respect to the nitrogen atom of the aminoacetyl part of the diphazine molecule increases considerably its anticholine action. This becomes particularly noticeable on comparison of the corresponding hydrochlorides (see table)

All the compounds studied showed most pronounced ability to block transmission in autonomic ganglia. Pentaphen and diphazine block conduction in the vagus in the same doses as tetraethylammonium [5, 2], arpenal and alpha-methyldiphazine – in doses which are 3-5 times smaller. Their alkyl iodide derivatives proved to be 20-30 times stronger than tetraethylammonium. The blocking action of the compounds under investigation on transmission in the superior cervical sympathetic ganglion proved to be markedly less pronounced than that of tetraethylammonium, although compounds with a quatenary nitiogen atom (alkyl iodides) approached tetraethylammonium in this respect. All the compounds studied thus show first of all, on intravenous injection, a blocking effect on conduction in the vagus, the, with increasing doses, a blocking effect on transmission in the superior cervical sympathetic ganglion, and in still larger doses exert a blocking effect on the muscarine-sensitive and nicotine-sensitive choline-reactive systems of the carotid sinus zones and the adrenals.

Some of the compounds studied, the methyl iodides in particular, may be of interest as substances exerting a selective blocking effect on conduction in the vagus. Consideration of the discovered correlations may facilitate further search for anticholine substances with selective action.

SUMMARY

Four groups of anticholine agents were studied. Each group consisted of three prepartions: the hydrochloride containing a tertiary nitrogen atom, and two alkyl iodides (methyl iodide and ethyl iodide) containing a quaternary nitrogen atom. All of the preparations with a quartemary nitrogen atom were several times more active than their tertiary analogue, blocking all peripherical choline-reactive systems. In respect to their sensitivity to these preparations, the choline-reactive systems may be grouped as follows: the most sensitive are the choline-reactive systems of the cardiac branches of the vagus nerve, then the superior cervical sympathetic ganglion, and last, the carotid sinus zones and the adrenals.

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THE PHARMACOLOGICAL CHARACTERISTICS OF THE CHOLINE REACTIVE BLOOD VESSEL SYSTEMS AND THE EFFECT UPON THEM OF SODIUM NITRITE AND BARIUM CHLORIDE*

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According to the views most widely held at present, the property possessed by sodium nitrite to weaken and by barium chloride to strengthen the contractions of smooth musculature is a result of its direct influence upon the muscle. However, it should be noted that this question has not been studied in recent years especially from the viewpoint of analyzing the basic biochemical processes underlying the act of smooth muscle contraction and studying those biochemical reactions which mediate the nerve impulse.

According to the literature [16, 20, 25], the essential action of the nitrites upon biochemical reactions consists in blocking those reactions which enable the organism to synthesize or utilize the adenotriphophoric acid which, according to present day concepts, is the basic source of the energy employed by the organism. It is known that adenotriphosphoric acid has to be present if these reactions are to take place and they are at the base of all muscular, including the smooth, contraction [4]. This does not warrant the conclusion that the nitrites act directly upon the muscle tissue itself. Thus brain tissue manifests a much greater sensitivity to lack of adenotriphosphoric acid than does muscle tissue [21] especially if this takes place during a period of oxygen deprivation. It should also not be forgotten that muscle tissue has a much greater capacity for the resynthesis of adenotriphosphoric acid than do other tissues as, for this purpose, aside from respiration, glycolysis may be utilized. In addition, muscular tissues use the energy derived from creatine phosphoric acid. Among the biochemical processes occurring in brain and nerve tissues attention should be drawn to the participation of adenophosphoric acid in the events of the cholinergic cycle, in particular the synthesis of acetylcholine [6, 27].

Description of the details of the influence exerted by barium chloride salts upon biochemical processes were not found in the literature available to us. Nevertheless, some facts speaking of their effect upon biochemical processes lying at the base of the activity of nervous tissue are known. Thus, attention is drawn to the weakening of intestinal response to barium chloride in the presence of thiamin avitaminosis [17]; the role played by the same vitamin B_1 in the normal activities of the nervous system, partially in the flow of the normal cholinergic processes, being well known. From the studies of A. I. Poskalenko [15], it can be seen that such fermentative poisons as fluorine or the cyanides, which upset carbohydrate metabolism, depress also the action of barium chloride and the influence of acetylcholine.

From this it may be assumed that barium chloride salts affect carbohydrate metabolism while those processes directly associated with the contraction of smooth musculature are related to the so-called adenylic cycle [4, 8].

Finally, it should be recalled that, according to the findings of N. V. Ermakova and G. G. Dyadyushi [3], preventing the access of barium chloride to the intramuscular portion of the innervation apparatus, markedly weakens the influence of barium upon frog striated muscle.

* Presented at conference commemorating the 30th anniversary of the death of Academician N. P. Kravkov. Ryazan, June 1954.

All these considerations give ground for the supposition that nitrites and barium salts act upon smooth muscles primarily by influencing the nerve tonus regulating the smooth musculature, partially through the medium of influences effected with the aid of the mediation of acetylcholine.

The experimental verification of this supposition is the basis of the present study.

EXPERIMENTAL METHODS

Experiments were performed upon the liver vessels (of a frog) isolated by the method of V. I. Berezin. The livers were taken from pond frogs of the autumn collection as well as from freshly-caught spring frogs. The pressure under which the solutions were perfused through the liver vessels varied in the various experiments between 2.5 and 3.5 cm water column readings.

At first, it was necessary to clarify the nature of acetylcholine action and other cholinergic substances upon the frog liver vessels.

An analysis of experiments performed by numerous investigators [11,14,18,19], permits the following deductions: frog liver vessels are sensitive to substances of the acetylcholine group (acetylcholine, carbocholine, arecholine and pilocarpine) and also of the nicotine group (nicotine, lobeline and cytisine). Also active are choline-sensitizing substances e.g., physostigmine and proserine. All of these have a vasoconstricting action. Nicotine, pilocarpine and, partially, physostigmine, after a two-hour perfusion, and an initial vasoconstriction, dilate the liver vessels. Of the cholinelytic substances we studied atropine and platyphylline. Atropine even when perfused for a long time narrows the liver vessels removing however the constricting capacities of arecoline and carbocholine. Platyphylline widens the frog liver vessels: occasionally, when rinsing platyphylline out, some constriction of the vessels is observed.

Analysis of this data permits the supposition that the liver vessel net has present within it N (nicotine - sensitive) choline-reactive systems, as classified by S. V. Anichkov [1]. In favor of this theory is the two phase action of nicotine as well as the sensitivity of the frog liver vessels to lobeline and cytisine. Also, attention is drawn to the two phase action of pilocarpine which is similar to its effect on the N choline reactive system in the cardiac vagal ganglion [9].

In order to verify that the vasodilating action of platyphylline is associated with a blockade of the M (muscarine sensitive) choline reactive system, we investigated the effect exerted by this substance upon the action of acetylcholine. Altogether 15 tests were performed. Marriott vessels were used to contain the perfusion fluid—a clear solution of normal Ringer's as well as a bitartrate solution of platyphylline. With the aid of a syringe, there was added to the perfusing fluid 0.2 cc of a 1:1000 solution of acetylcholine.

Platyphylline did not remove the acetylcholine action from the frog liver vessels. Thus, when introduction of acetylcholine during perfusion of the liver with Ringer's solution diminished, on the average, the number of drops flowing out of the liver veins by 63%, against a preliminary rinsing of the liver with platyphylline (1:2000), when the dilatation of the vessels had reached a stable extent, under the influence of acetylcholine the number of drops decreased an average 83%.

The ability of atropine to remove the vasoconstricting effects of arecoline and carbocholine can be completely attributed to the influence exerted by atropine upon the N choline sensitive vessel system, at least as far as there is, for example, data on the ability of atropine to block them in the sympathetic ganglia [23,24]. It is also known that arecoline can stimulate also the N choline sensitive systems as, e.g., striated muscles [2]. The ability of atropine to act like acetylcholine in constricting the frog liver vessels also is contrary to the concept of it blocking the M choline sensitive systems and is more to be explained by the anticholinesterase action of the atropine which is a property more or less common to all substances capable of competing with acetylcholine because of their choline sensitive structure [5]. This anticholine esterase action may be the reason for platyphylline reenforcing the acetylcholine action in our experiments. We have already pointed out that, according to the literature, typical anticholinesterase substances such as physostigmine and proserine* also exert a vasoconstricting influence upon frog liver vessels. At this point it should be noted that, according to many opinions [7, 10, 11, 12], the regulation of vasomotor tone, aside from central influences, is mediated by a nervous mechanism present in the vessel walls. In this manner, the analysis of the literature and our own data leads us to the conclusion that the choline reacting vessel systems in the frog liver belong to the nicotine sensitive group.

* Russian trade name.

According to Japanese investigators [22], acetylcholine exerts a nicotine like action upon the vessels in the frog rear limbs.

In order to determine whether the vasodilating action of sodium nitrite is a result of a blockade of the choline reactive systems, we utilized the antagonism between proserine and the cholinelytic action of certain pharmacological substances. As is known, this action is regarded as a manifestation of the anticholinesterase influence of proserine as a result of which the competing relations of the spontaneously forming acetylcholine and cholinelytic agents within the tissues are altered in their similarities to their choline sensitive structure: the sensitivity of the latter becoming restored.

First, we had to investigate whether we could demonstrate this antagonism in our experimental object. We studied the influence of proserine (1:4000 and 1:2500) upon vasodilation produced by large concentrations of the nicotine like substance anabasine (1% and 1.5%). It has been established that large concentrations of nicotine like substances block N choline sensitive systems.

As can be seen from Table 1, in the frog liver vessels, proserine manifests the capacity of removing the blockade of the N choline reactive systems.

TABLE 1

Influence of Proserine Upon the Vasodilating Action of Anabasine (experiment performed April 2, 1952)

Time from start of exp. (min)	Perfusion fluid	Drops per minute
0—6	Normal Ringer solution	51. 51. 51. 51. 51. 51
7—15	Anabasine 1 : 100	55. 54. 55. 56. 57. 58. 58. 60. 62
16—21	Anabasine 1 : 100 + Proserine 1 : 2500	59. 57. 51. 49. 49. 49
22—101	Rinsing with Ringer solution	—
102—105	Ringer solution	50. 51. 50. 50
106—120	Anabasine 1 : 100	51. 51. 52. 52. 54. 53. 55. 55. 56. 58. 57. 57. 61. 60

In the succeeding 40 experiments we investigated the influence of proserine (1:4000 and 1:2000) upon the vasodilating action of sodium nitrite (1:20,000 and 1:10,000). The protocol of one such experiment of this series is shown in Table 2.

As A. A. Nikulin [14] has shown, at a pH of 8.3 sodium nitrite constricts the frog liver vessels. But as we have shown in our experiments neither nitrite nor its mixture with proserine altered the reaction of Ringer's solution medium. Hence, the vasodilating action of sodium nitrite must be associated with a blockade of the N choline reactive structures in the frog liver vessels.

To analyze the effect of barium chloride upon the frog liver vessels, we decided to observe the influence dimedrol* had upon the vasoconstricting power of barium as dimedrol had been shown by M. D. Mashkovsky and S. S. Liberman [13] to have the ability of blocking N choline reactive systems. To confirm this ability of dimedrol to block the N choline reactive systems in the frog liver vessels we decided to test the influence of dimedrol upon the vasoconstricting power of anabasine sulphate (10 experiments): taking for this purpose 0.5 cc of a 6% anabasine solution, prepared in Ringer solution and introduced directly into the stream of the perfusing fluid.

It became apparent that dimedrol dilated the frog liver vessels. The vasoconstricting action of anabasine was not manifested if the liver vessels had been first perfused by dimedrol which dilated the liver vessels to a constant width.

To convince ourselves that the vasodilating and cholinelytic action of dimedrol is associated with its

* Russian trade name.

influence upon the choline reactive systems and is not a direct effect upon the biochemical processes underlying the act of muscle contraction, in this series of experiments, as with those done with sodium nitrite, we tested in 10 experiments the influence of proserine upon the vasodilating action of dimedrol (Table 3).

We see that proserine removes the dimedrol action; it follows that the vasodilating effect of dimedrol is associated with a blockade of the N choline reactive systems in the vessels of the frog liver.

After this we investigated the influence of dimedrol upon the vasoconstricting action of barium chloride (20 experiments). 0.5 cc of a 6% solution of barium chloride introduced into the perfusion fluid (Table 4).

TABLE 2

Influence of Proserine Upon the Vasodilating Action of Sodium Nitrite (February 22, 1952)

Time from start of exp. (min)	Perfusion fluid	Drops per minute
0—5	Normal Ringer	42. 41. 42. 41. 42
6—26	sodium nitrite 1: 10 000	42. 43. 43. 44. 46. 46. 46. 46. 47. 48. 47. 49. 49. 48. 49. 50. 51. 51. 50. 51. 51
27—32	Sodium nitrite 1: 10 000 + Proserine 1 : 2 000	51. 49. 44. 38. 37. 35
33—67	Rinsing with Ringer	—
68—71	Ringer solution	42. 41. 41. 41
72—82	Sodium nitrite 1 : 10 000	41. 42. 43. 46. 47. 48. 49. 49. 50. 50. 50
83—94	Sodium nitrite 1: 10 000 + + proserine 1: 2 000	49. 42. 46 43. 40. 38. 37. 36. 32. 27. 23. 15
95—136	Rinsing with Ringer solution	—
137—140	Ringer solution	35. 35. 36. 35

TABLE 3

Influence of Proserine Upon the Vasodilating Action of Dimedrol (experiment performed January 27, 1953)

Time from start of exp. (min)	Perfusing fluid	Drops per minute
0—5	Normal Ringer solution	46. 45. 45. 46. 45
6—10	Dimedrol 1 : 500	46. 45. 45. 45. 45
11—39	Same	—
40—43	» »	52. 52. 52. 52
44—49	Dimedrol 1 : 500 + Proserine 1 : 2 000	48. 45. 40. 39. 39 37
50—79	Rinsing with Ringer	—
80—83	Ringer solution	45. 46. 46. 46

These experiments show that dimedrol blocks the vasoconstricting action of barium chloride. It follows that the action of barium chloride is associated with stimulation of the N choline reactive systems in the vessels of the frog liver.*

TABLE 4

Influence of Dimedrol Upon the Vasoconstricting Action of Barium Chloride (experiment performed February 5, 1953)

Time from start of exp. (min)	Perfusing fluid	Drops per minute
0—4	Normal Ringer solution	43. 43. 43. 43
5—14	Barium chloride	52. 44. 41. 38 37 38. 38. 39. 38 39
15—18	Dimedrol 1 : 500	38. 40. 39. 38
19—47	Same	--
48—51	» »	47. 48 48. 48
52—64	Barium chloride	48. 48. 49. 48. 48 48. 48 48. 48 48 48. 48 48
65—130	Rinsing with Ringer solution	—
131—134	Ringer solution	43. 42. 42. 42
135—145	Barium chloride	46. 41. 39. 38. 38. 38. 37. 37. 35. 36. 35

EXPERIMENTAL RESULTS

Pharmacological analysis shows that vasomotor tone regulation in the frog liver vessels is associated with cholinergic mechanisms. The choline reactive vessel systems in the frog liver belong to the nicotine sensitive group (S. V. Anichkov classification). The vasodilating action of sodium nitrite appears to be associated, apparently, with the influence of this substance upon cholinergic processes and not with a direct interference with the chemodynamics of the smooth blood vessel musculature as has been supposed.

The same remarks apply to the vasoconstricting action of barium chloride.

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* In the same year of 1954 when we reported our studies, Levy and Michel-Ber [26] showed that barium chloride acts on the bowel in a ganglionic manner.

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ONCOLOGY

SIMILARITIES AND DIFFERENCES OF SPECIFIC ANTIGENS OF CANCER IN MAN

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In the course of immunologic studies on malignant tumors sufficient facts have been collected to suggest their practical application for the diagnosis, prophylaxis and therapy of cancer. Of particular significance in this connection is the question of similarity or difference of antigens determining the specificity of cancerous tumors in man; this question has not, however, up to now received a definitive answer.

V. V. Gorodilova and L. V. Shershulskaya [2], using the method of anaphylaxis with desensitization, found that cancerous tumors in man with different localization (stomach, liver, breast, uterus, ovaries) contained both specific antigens common to all the tumors and separate components specific for each particular tumor. Similar results were also obtained by V. A. Korenevskaya [3] who investigated specific antigens from 11 varieties of malignant brain tumors of neuroectodermal and meningo-vascular type. As this author notes, the antigenic similarity of the tumors studied has, as a rule, greater constancy than their differences.

The work of V. V. Gorodilova and V. B. Freiman [1] established that the specific antigen characteristics of tumors are not limited by the species of the host-carrier of the tumor, but extends to tumors in animals of other species. These authors found antigenic similarity between cancerous breast tumors of man and mouse. In addition to the similar components detected in the cancerous tissues of man and mice dissimilar components were also found in these tissues.

Other data were obtained by A. K. Saakov [7] who used absorbed anti-tumor sera and found only one specific antigen common to cancer of the uterus, cancer of the stomach and cancer of the lung in man.

Analogous results were obtained by I. Makari and M. Huch [9], using the Schultz-Dale reaction (anaphylaxis with desensitization on isolated organs of the guinea pig). In their studies on sera from patients the authors found a soluble antigen common to all forms of cancer, independent of its type or site of origin.

In contradistinction to the investigations described above, we [4] found, using anti-tumor sera freed by absorption from antibodies to normal organs, that no single antigen specific for all tumors in man exists. Each of the three cancerous tumors studied by us contained its particular specific antigen which was absent in the other two. This material naturally could not be considered adequate for judging to what extent the observed fact could be applied to other tumors or for answering the question whether each tumor was characterized by specific properties particular to it alone or whether tumors could possess similar antigens. Further work was needed to clarify these problems; the results are presented in the present communication.

The material for the present investigation was formed by 45 cancerous tumors in man, of different localization and structure; moreover, the patients belonged to different blood groups. Comparative studies were made on the antigenic properties of tumors of the stomach, breast, lung, ovary, prostate, liver and metastases in the liver from cancer of the stomach, lung, ovary, esophagus, cecum, colon and others.

EXPERIMENTAL METHODS

The experimental methods for absorption and complement fixation as well as the use of specific anti-tumor sera No. 388 and No. 114 (against tumor No. 1 – liver metastasis of cancer of the colon), No. 528 and No. 990 (against tumor No. 2 – liver metastasis of cancer of the gall bladder), No. 49, No.1758 and No. 1934 (against tumor No. 3 – primary cancer of the liver) have already been described by us in detail in previous communications [4-5].

Sera organ-specific to liver and spleen in man were used for control simultaneously with anti-tumor sera.

Antigens for absorption and complement fixation were prepared from fresh and formalin-or glycerine-preserved tumor tissues. Formalin-or glycerine-treated tissues were first freed from the preserving fluid by washing in running tap-water or by dialysis in physiologic solution. Absorption of sera by fresh tumor tissue could only be achieved successfully when the material had not been stored for long, so that material obtained at operation gave the best results. The tissue in these cases was macerated and washed immediately before the experiment.

Control antigens were prepared from fresh spleen and liver tissue taken from healthy persons who died accidentally.

EXPERIMENTAL RESULTS

In Table 1 is presented a typical protocol of an experiment on comparative study of water-salt extracts from tumors by means of complement fixation reaction. The table shows that anti-tumor serum No. 1758 gives a positive complement fixation reaction with extract of tumor No. 3, with respect to which this serum was obtained, and also with one of the other tumors under investigation, viz. No. 12. This serum did not, however,

TABLE 1

Comparative Studies on Specific Antigenic Properties of Tumors Using the Complement Fixation Method

Serum	Dilution of Sera	Antigens from Tumors										Antigens from spleen	Serum Control
		№ 1	№ 3	№ 4	№ 5	№ 7	№ 8	№ 9	№ 10	№ 11	№ 12		
Anti-tumor No. 1758 (against tumor No.3)	1:20	–	++++	–	–	–	–	–	–	–	++++	–	–
	1:40	–	+++	–	–	–	–	–	–	–	+++	–	–
	1:80	–	±	–	–	–	–	–	–	–	±	–	–
Anti-spleen No. 57	1:80	–	–	–	–	–	–	–	–	–	–	++++	–
	1:160	–	–	–	–	–	–	–	–	–	–	++++	–
Antigens control		–	–	–	–	–	–	–	–	–	–	–	

Conventional signs: ++++, +++, ++, +, ± different degrees of positive reaction; – negative reaction.

Note. Tumor No. 1 – liver metastasis of cancer of the cecum; No. 3 – primary cancer of the liver; No. 4 – liver metastasis of cancer of the stomach; No. 5 – liver metastasis of cancer of the bile duct; Nos. 7 and 8 – liver metastasis of cancer of the stomach; No. 9 – primary cancer of the liver; No. 10 – liver metastasis of cancer of the ovary; No. 11 – liver metastasis, primary site unknown; No. 12 – liver metastasis of cancer of the stomach.

react with the water-salt extracts of tumors No. 1, 4, 5, 7, 8, 9, 10 and 11; this indicates the absence in these of antigens identical with antigens of tumors No. 3 and No. 12. The anti-tumor serum used in the experiments was specific and did not react with antigen from normal organ (spleen). The working dose of spleen antigen is shown to be correct by the positive reaction obtained with the same dose of spleen antigen and the corresponding organ-specific serum.

TABLE 2

Comparative Studies on Specific Antigenic Properties of Tumors Using the Absorption Method

Serum	Tissue used for Serum Absorption	Dilution of Sera	Antigens from Tumors								Antigen from spleen	Sera control
			№ 1	№ 4	№ 6	№ 7	№ 8	№ 9	№ 10	№ 11		
Antitumor No. 388 (against tumor No. 1)	Spleen	1:80	++++	++++	++++	–	–	–	–	–	–	–
		1:160	++++	++++	++++	–	–	–	–	–	–	–
		1:320	++++	++++	+++	–	–	–	–	–	–	–
	Tumor № 1	1:80	–	–	–	–	–	–	–	–	–	–
		1:160	–	–	–	–	–	–	–	–	–	–
		1:320	–	–	–	–	–	–	–	–	–	–
	Tumor № 4	1:80	–	–	–	–	–	–	–	–	–	–
		1:160	–	–	–	–	–	–	–	–	–	–
		1:320	–	–	–	–	–	–	–	–	–	–
	Tumor № 6	1:80	–	–	–	–	–	–	–	–	–	–
		1:160	–	–	–	–	–	–	–	–	–	–
		1:320	–	–	–	–	–	–	–	–	–	–
	Tumor № 7	1:80	++++	++++	++++	–	–	–	–	–	–	–
		1:160	++++	++++	++++	–	–	–	–	–	–	–
		1:320	++++	++	+	–	–	–	–	–	–	–
	Tumor № 8	1:80	++++	++++	++++	–	–	–	–	–	–	–
		1:160	++++	++++	+++	–	–	–	–	–	–	–
		1:320	++++	++	+	–	–	–	–	–	–	–
	Tumor № 9	1:80	++++	++++	++++	–	–	–	–	–	–	–
		1:160	++++	++++	+++	–	–	–	–	–	–	–
		1:320	++++	+++	+	–	–	–	–	–	–	–
	Tumor № 10	1:80	++++	++++	++++	–	–	–	–	–	–	–
		1:160	++++	++++	+++	–	–	–	–	–	–	–
		1:320	++++	+++	±	–	–	–	–	–	–	–
	Tumor № 11	1:80	++++	++++	++++	–	–	–	–	–	–	–
		1:160	++++	++++	+++	–	–	–	–	–	–	–
		1:320	++++	+++	++	–	–	–	–	–	–	–
Antigen Control			–	–	–	–	–	–	–	–	–	

Conventional signs the same as in Table 1.

Note. Tumor No. 1 – liver metastasis from cancer of the cecum; Nos. 4, 6, 7 and 8 – liver metastasis from cancer of the stomach; No. 9 – primary cancer of the liver; No. 10 – liver metastasis of cancer of the ovary; No. 11 – liver metastasis, primary site unknown.

TABLE 3

Results of Comparative Studies on Specific Antigenic Properties of Tumors in Man

No. of tumor	Localization of tumor	Blood group	Number of anti-tumor serum						
			Against tumor No. 1		Against tumor No. 2			Against tumor No. 3	
			388	114	528	990	49	1758	1934
1	Liver metastasis of cancer of the cecum	0	+	+	–			–	–
2	Liver metastasis of cancer of the bile duct	0	–	–	+	+	–	–	–
3	Primary cancer of the liver	B	–	–	–	–	+	+	+
4	Liver metastasis of cancer of the stomach	B	+		–	–	–	–	–
5	Liver metastasis of cancer of the bile duct	0	+			–		–	–
6	Liver metastasis of cancer of the stomach	0	+		–	–		–	
7	Liver metastasis of cancer of the stomach	0	–		–	–		–	–
8	Liver metastasis of cancer of the stomach	0	–		–	–		–	–
9	Primary cancer of the liver	A	–		–	–		–	–
10	Liver metastasis, of cancer of the ovary	A	–		–	–	–	–	–
11	Liver metastasis, primary site unknown	0	–		–	–		–	–
12	Liver metastasis of cancer of the stomach	A	–		–	–		+	+
13	Liver metastasis of cancer of the uterus		–		–		–	–	–
14	Liver metastasis of cancer of the stomach	A	–		–		–	–	–
15	Liver metastasis of cancer of the stomach	B	–		–		–		–
16	Liver metastasis of cancer of the lung	A	–		–				–
17	Cancer of the stomach		–	–	–				–
	Liver metastasis	B	–	–					
	Lymph gland metastasis		–	–					
18	Cancer of the lung	A	+		–				–
19	Cancer of the lung		–		–				–
20	Liver metastasis of cancer of the lung		–		–				–
21	Cancer of the stomach	A	–	–	–				–
	Liver metastasis of cancer of the stomach		–	–					
22	Cancer of the stomach		–		–				–
23	Cancer of the stomach	0	–		+				–
24	Cancer of the stomach		–						–
25	Cancer of the ovary	A							–
26	Liver metastasis of cancer of the lung	A	–						
27	Cancer of the lung	B	–	–					
28	Cancer of the prostate	0	–	–					
29	Liver metastasis of cancer of the stomach	A			–	–	–	–	–
30	Liver metastasis of cancer of the cervix	0			–		–	–	–
31	Cancer of the breast				–		–		
32	Cancer of the breast	0	–						
33	Cancer of the esophagus growing into bronchus	0		+					
	Liver metastasis			+					
34	Liver metastasis of cancer of the pancreas	0		–					
35	Lung metastasis of cancer of the stomach	A		–					

(Table 3 cont'd)

No. of tumor	Localization of tumor	Blood group	Number of anti-tumor serum						
			Against tumor No. 1		Against tumor No. 2		Against tumor No. 3		
			388	114	528	990	49	1758	1934
36	Cancer of the breast	B		–					
37	Skin (chest) metastasis of cancer of the breast	B		–					
38	Liver metastasis of cancer of the stomach	A					–	–	–
39	Liver metastasis of cancer of the colon	B		–					
40	Liver and spleen from patient with leukemia	A		–					
41	Liver metastasis of cancer of the stomach	B		–					
42	Cancer of the stomach	B		–					
43	Cancer of the lung	AB	–						
44	Cancer of the stomach	0		–					
	Liver metastasis			–					
45	Cancer of the stomach	A		+					
	Lymph gland metastasis			+					

Conventional signs: + positive complement fixation and absorption reaction results; – negative results.

These experiments, on the one hand, confirm the fact established by us earlier [4] concerning the difference of specific tumor antigens and, also, demonstrate that there are tumors which are characterized by the presence of similar specific antigens (tumors No. 3 and No. 12).

Another confirmation of this fact came from investigation of tumor antigens by means of specific absorption. Table 2 presents a protocol of experiment on absorption of anti-tumor serum No. 388 (against tumor No. 1) by different samples of tumor tissues. The experiment included tumor tissues No. 4 and No. 6, whose water-salt extracts, like that of tumor No. 1, gave a positive complement fixation reaction with anti-tumor serum No. 388 and tumor tissues Nos. 7, 8, 9, 10 and 11 whose water-salt extracts gave negative complement fixation reactions (see Table 2, absorption of serum by spleen tissue). Absorption of serum No. 388 by tumor tissues Nos. 1, 4 and 6 led, as can be seen from Table 2, to complete loss of its ability to react with water-salt extracts from tumors Nos. 1, 4 and 6. Conversely, tumor tissues Nos. 7, 8, 9, 10 and 11, whose water-salt extracts gave a negative complement fixation reaction, could not extract from the serum anti-tumor antibodies in the course of absorption. In the course of absorption the serum retained its ability to react with water-salt extracts of tumors Nos. 1, 4 and 6.

Thus, the experiments performed by us on specific absorption provided new proofs in favor of the existence of tumors both differing in their specific antigenic properties and similar with respect to specific antigen.

As has been mentioned above, comparative studies were carried out on 45 cancerous tumors of man. The results of all these investigations are summarized in Table 3 which shows that of 32 tumor antigens tested with sera No. 388 and No. 114 (against tumor No. 1) only 6 antigens (Nos. 4, 5, 6, 18, 33, 45), as well as antigen No. 1, gave a positive reaction, i. e. proved to be similar to antigen No. 1.

The majority of the antigens, however, reacted negatively thus demonstrating qualitative differences between the antigenic properties of these 27 tumors from those of tumor No. 1 and tumors Nos. 4, 5, 6, 18, 33 and 45 similar to No. 1.

Of 26 tumor antigens tested with sera Nos. 528 and 990(against tumor No. 2) only one antigen, No. 23, like antigen No. 2 which was used for the serum, reacted positively, the remaining 24 antigens gave a negative result.

Twenty-nine tumor antigens were studied with anti-tumor sera Nos. 49, 1758 and 1934 (against tumor No. 3). Apart from antigen from tumor No. 3, a positive reaction was found only with antigen from tumor No. 12.

It must be noted that sera of one series which were obtained from different rabbits but were immunized by the same tumor antigen (e. g. sera Nos. 49, 1758, 1934) behaved consistently in the experiments, in other words they all gave either a positive or a negative reaction when treated with the antigen being tested.

As can be seen from Table 3, attempts to establish some relationship between the ability of a tumor antigen to enter into immunologic reaction with one or other anti-tumor serum and localization of this tumor proved unsuccessful. Thus, for example, tumors Nos. 18, 33 and 45 gave a similar positive reaction with sera Nos. 388 and 114 despite the fact that they differed in localization. On the other hand, tumors with similar localization behaved differently in their reactions, e. g. tumors Nos. 3 and 9 reacting with sera Nos. 1758 and 1934.

This ability of tumors to enter into reaction did not depend on the host's blood group either, and hence did not depend on the group antigens present in the tumors.

It should be noted particularly that primary tumors and their metastases obtained from one and the same patient possessed similar specific antigenic properties. If the original tumor entered into immunologic reaction with one or other serum, its metastases also reacted positively, and vice versa (e.g. tumor No. 17 or No. 45, see Table 3).

We did not observe a single case of divergence in the behavior of primary tumor and its metastases.

Unlike the data of L. A. Silber and his collaborators, A.K. Saakov, Makari and Huch and others, these investigations have shown that no single antigen, specific for all cancerous tumors of man, exists. Some tumors are qualitatively different as regards antigens and have no common specific "cancer" antigens; other tumors, on the contrary, are characterized by the presence in them of similar, and possibly identical, specific antigens.

This has also found support in the work of P. N. Kosyakov and N. I. Kuznetsova [6].

In connection with the results obtained by us it is of interest to note the data reported by J. Graham and R. Graham [8], according to which patients with cancer show antibodies which react with antigen from tumors of other patients.

The same authors, however, report that in some, more rare, cases tumors did react similarly with one and the same serum, i. e. showed similar antigenic properties.

The question concerning the causes of similarity of specific antigens in some cancerous tumors in man and, conversely, of differences in others remains unanswered and requires further study.

SUMMARY

The authors employed reactions of complement fixation and specific absorption for comparative study of the antigenic properties of tumors in man. As a result of these experiments it was established that there is no single antigen which is specific for all tumors of man. Certain tumors are qualitatively different and do not have common specific "cancer" antigens. On the contrary, other tumors are characterized by the presence of similar specific antigens.

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THE SEARCH FOR ANTI-TUMOR PREPARATIONS AMONG THE DERIVATIVES OF BENZIMIDAZOLE

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Benzimidazole is an analogue of purine and differs from the latter in that instead of a pyrimidine ring it contains a benzene ring.

The benzimidazole ring plays an important part in metabolism (enters into the composition of vitamin B_{12}, participates in the synthesis of thymine nucleosides).

On the other hand Woolley [1] has shown that benzimidazole added to the nutrient medium inhibits the growth of yeast and of some bacteria. The inhibitory action of benzimidazole can be abolished by adenine or guanine.

Taking these data into consideration with respect to the intensive synthesis of nucleic acids in malignant tumors, L. F. Larionov and L. L Malyugina [2] undertook the study of the effect of benzimidazole and some of its derivatives on tumors in animals.

The compounds were synthesized by O. F. Ginzburg and L. S. Efros (1952-1956) in the Laboratory of Organic Dye-stuffs (Director B. A. Porai-Koshits) of the Lensovet Technological Institute, Leningrad.

However, none of the derivatives of benzimidazole obtained (with substituents in position 2, which included methyl, phenyl, benzyl and pyridyl) showed any activity against transplanted tumors in rats and mice.

The problem in the present work was to investigate the possibility of using benzimidazole as a carrier of an anti-tumor active chemical group and as its "conductor" into the tumor.

METHODS AND RESULTS

Six derivatives of benzimidazole, prepared by O. F. Ginzburg, were studied; these were called "dimezoles" and included dimezole-7 (2-chloro-methylbenzimidazole), dimezole-8 (1-β-hydroxyethylbenzimidazole), dimezole-9 (1-β-chloroethyl-2-chloromethylbenzimidazole), dimezole-12 (1-β-chloroethyl-2-benzylbenzimidazole), dimezole-13 [1-β-chloroethyl-2-bis-(β-chloroethyl)-aminomethylbenzimidazole] and dimezole-14 [2-bis-(β-chloroethylaminomethylbenzimidazole)].

Investigation of the benzimidazole derivatives was started with determination of their toxicity. Experiments with single administration of the substances to rats (80) showed that dimezoles 11, 12 and 13 were non-toxic, since doses of 2.5-2 and 0.5 g per 1 kg body weight did not cause death of the animals. Dimezole-7 is slightly toxic – 30% of the rats succumbed to a dose of 1.5 g/kg body weight. Dimezole-9 is rather more toxic: 80% of rats succumbed to a dose of 0.5 g/kg. According to the data of L. F. Larionov and L. L. Malyutina [2] the lethal dose of dimezole-8 (for mice) is 1.5 g per 1 kg. Dimezole-14 is the most toxic, the 100% LD for it being 0.5 g/kg and the 50% LD about 0.1 g/kg.

The anti-tumor properties of the preparations were studied on transplanted tumors: Ehrlich's, sarcoma 45 and Gueren's carcinoma. A total of 370 mice and 500 rats was used.

Experiments were always performed on measurable tumors. The tumors were measured with calipers in three directions. The arithmetic mean was calculated from the figures obtained and this served as the mean diameter of the tumor. The results were evaluated by percentage inhibition of tumor growth. The data were treated statistically by the modified method of Student as described by V. I. Romanovsky [3] (the difference was considered significant if $r = 0.955$).

Experimental results are summarized in the table.

The Effect of Benzimidazole Derivatives on Transplanted Tumors

Preparation	Tumor Strain	Dose in mg/kg	No. of animals	% Inhibition	Criterion of signif.
Dimezole-7	Ehrlich's tumor	275	20	—12	0.397
	Sarcoma 45	275	25	21	0.730
Dimezole-8	Ehrlich's tumor	300	15	20	0.661
Dimezole-9	Ehrlich's tumor	50	120	40	0.987
	Sarcoma 45	50—60	140	18	0.379
Dimezole-12	Sarcoma 45	300	30	30	0.979
		250	30	—30	0.723
		300	—	—20	0.838
Dimezole-13	Sarcoma 45	400	25	45	0.927
Dimezole-14	Ehrlich's tumor	5—7	145	40	0.998
	Sarcoma 45	3.5	60	95.1	0.997
	Guerin's carcinoma	3.5	30	70	0.979

Note. All preparations were administered per os except dimezole-8 which was given intraperitoneally.

The table shows that neither dimezole-7 nor dimezole-8 exerted any appreciable effect on the growth of Ehrlich's tumor. Nor does dimezole-7 inhibit the growth of sarcoma 45.

Dimezole-9 produces noticeable inhibition of the growth of Ehrlich's tumor but not of sarcoma 45.

Dimezole-12 inhibited the growth of sarcoma 45 in one experiment by 30% but in other experiments produced statistically unreliable stimulation of the growth of the same tumor.

Dimezole-13 failed to show anti-tumor activity since the inhibition obtained in 45% proved to be non-significant.

Definite anti-tumor activity was found in dimezole-14. In daily doses of 3.5 mg/kg or 15-20 mg/kg given every 72 hours, this preparation not only produced strong inhibition of the growth of sarcoma 45 (by 95%) but also caused resolution of this tumor in 34% of the rats if the tumor weighed about 1 g at the beginning of treatment. No such resolution was observed on treating rats with large tumors (about 4 g) even when toxic doses were used, but tumor growth was inhibited by 90%.

Anti-tumor activity of dimezole-14 is also seen in the case of other transplanted tumors. It inhibits the growth of Ehrlich's tumor in mice by 40% and of Gueren's rat carcinoma by 70%.

Rats treated with dimezole-14 showed a $1^1/_2$-2-fold decrease in the weight of the spleen as compared to control animals.

Investigation of the effect of dimezole-14 on hemopoiesis was carried out on 10 rabbits; dimezole-14 was introduced into the stomach for 8 days. Daily leucocyte counts and determinations of the leucocyte formula were done. In addition, at the beginning and end of the experiment examination of bone marrow (puncture of the tibia) was performed, and when the animals were sacrificed the hemopoietic organs were examined microscopically (bone marrow, spleen, thymus and lymph nodes). The results of these experiments showed that dimezole-14 under these conditions had a weak depressing effect on hemopoiesis.

Of the 6 derivatives of benzimidazole investigated considerable anti-tumor activity was thus found in one preparation – dimezole-14 – which has one bis-(β-chloroethyl)-amine group linked with the ring by a CH_2 group.

The results of this investigation suggest that further search for anti-tumor preparations in this group of compounds is promising.

SUMMARY

The authors studied the effect of 6 derivatives of benzimidazole on the transplanted tumors of mice and rats. Pronounced anti-tumor activity was revealed in dimezole-14 [2-bis-(β chlorethylaminomethylbenzimidazole)]. Dimezole-14 not only inhibits the growth of sarcoma 45, Gueren's carcinoma and Ehrlich's tumor, but even causes resolution of sarcoma 45 in certain rats. This shows that the search of anti-tumor preparations among derivatives of benzimidazole has important prospects.

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EXPERIMENTAL BIOLOGY

THE EFFECT OF SEXUAL STIMULATION ON REGENERATION AND COMPENSATORY HYPERTROPHY IN THE MOUSE OVARY

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We showed in 1952 that complete regeneration of mammalian ovaries is possible only under certain conditions. Regeneration always occurs after removal of one entire ovary and half of the other. The remaining half ovary regenerates to form a complete and functional gonad. We also showed that administration of bromine and caffein affects the compensatory hypertrophy and regeneration, evidently as a result of the action of these drugs on the central nervous system.

G. V. Kharlova showed in 1956 that mechanical stimulation of the conducting pathways caused regeneration of rat ovaries.

Thus, ovarian regeneration and hypertrophy depend on the condition of the animal and its different organs. An investigation is therefore required to find the effect of various substances' function, on ovarian regeneration and hypertrophy. In particular, it is essential to discover the effect of sexual stimulation.

For this purpose, the following experiments were carried out.

The mice were divided into two sets. Each set consisted of two experimental and one control group. The first group consisted of normal unoperated mice. The second group consisted of mice from which the left ovary had been removed in order to obtain compensatory hypertrophy of the right ovary. The third group was composed of mice from which the whole of one (and half of the other) ovary had been removed.

The first set of mice, apart from the operations described above, were not given any experimental treatment.

In the second set, the mice of all three groups were stimulated sexually by placing them in a cage together with male mice, but separated from them by a wire mesh partition (Fig. 1).

The experiment lasted for six weeks. The results are shown in the table.

In the experiments in which females were kept together with the males, the nonoperated normal mice showed an increase in both absolute and relative ovary weight from 4.3 mg (0.019%) in the control mice to 5.2 mg (0.021%) in the stimulated animals. Histological investigation showed an increase in the number of ripening follicles and corpora lutea in comparison with the controls in whom no reduction in the primary follicles was observed.

Thus, the presence of males on the other side of the wire partition induced an effect in the ovaries.

After removal of one ovary there was a greater compensatory hypertrophy in females kept with males than in the controls. In the stimulated animals, the ovary weight reached 9.5 mg (0.045%), that is, an increase of two times; in females segregated from the males, the weight of the remaining ovary reached only 8.1 mg

(0.034%), i. e., it exceeded the weight of a nonoperated mouse by 70%. Histological investigation showed that there was compensatory hypertrophy in mice of both sets, but that it was more clearly shown in those animals which had been kept together with the males. In these latter animals there was an increased ripening of follicles and formation of corpora lutea, which filled almost the whole of the ovary. There was also a small increase in the number of primary follicles.

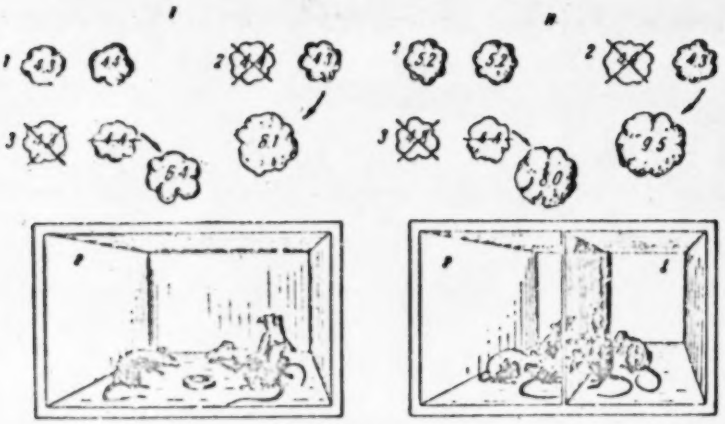

Fig. 1. Arrangement of experiments on regeneration of mouse ovaries with (II) and without (I) sexual stimulation.

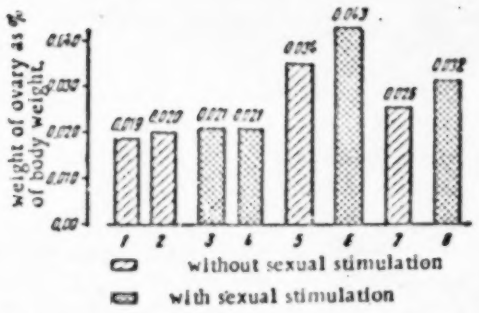

Fig. 2. The effect of experimental conditions on regeneration and compensatory hypertrophy of the ovary: with and without sexual stimulation.

From these results it can be seen that sexual stimulation causes an increase in ovarian compensatory hypertrophy.

In mice of the third group, in whom one whole and one half ovary had been removed, there was a complete regeneration of the remaining half ovary in both the first and the second sets of mice.

In the first set of mice, kept separate from the males, the relative weight of a single entire ovary was 0.020%, while that of the residual portion of the left ovary was 0.026%, i. e., 30% higher.

In the second set of mice, kept with the males, the regeneration of the traumatized ovary was more rapid. At the end of the experiment the weight of the residual portion of the left ovary had attained a weight of 8 mg (0.032%), i. e. it exceeded by one and a half times the weight of an ovary of a nonoperated animal. A histological investigation of the regenerating ovaries showed an increase in the number of corpora lutea and a normal number of follicles.

It can be seen by comparing the increase in weight in the nonoperated animals in the two sets that the presence of the males stimulates ovarian proliferation. In addition it can be seen that compensatory hypertrophy and regeneration are also stimulated. These two processes are better shown in the second set of mice, kept with the males, as can be seen by comparing the relative increase in ovary weight in the second and third groups with that in the first.

TABLE

Ovary Weight in Sexually Stimulated Mice (Kept Together with Males and Separated by a Wire Partition)

Group	No stimulation						Sexual stimulation						Probability of Chance occurrence of diff. between stim. and unstim. groups.
	No. of mice	Average wt. of mice in mg.	Average ovary wt. in mg.		Ovary wt. as % of body wt.		No. of mice	Average wt. of mice in mg.	Average wt. of ovary in mg.		Ovary wt. as % of body wt.		
			Right	Left	Right	Left			Right	Left	Right	Left	
First group – normal mice	20	21 590	4.3	4.4	0.019	0.020	22	20 900	5.2	5.2	0.021	0.021	0.027
Second group – left ovary removed	19	23 200	8.1	—	0.034	—	18	22 168	9.5	—	0.043	—	0 002
Third group – right ovary and half left ovary removed	20	22 620	—	6.4	—	0.026	18	22 450	—	8.0	—	0.032	0.012

We may thus conclude that in mice kept with males ovarian compensatory hypertrophy and regeneration proceed more intensively.

SUMMARY

Sexual stimulation, which consists in the influence of males on females which are separated by a wire partition, affects the process of growth of the ovaries in mice, as well as their compensatory hypertrophy and regeneration. Compensatory hypertrophy and regeneration of half of the ovary which remained intact after complete removal of the other, is more intensive when females are in one cage with males.

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THE EFFECT OF NUTRITION AND SCIATIC NERVE SECTION ON REGENERATION OF THE MUCOUS MEMBRANE OF THE SMALL INTESTINE IN THE CRESTED NEWT

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Physiological regeneration of the epithelium of the small intestine has been studied chiefly in mammals [1, 3, 4, 5, 6]. Seasonal changes in the frog intestine have been studied only by A. M. Vasyutochkin [2], who found that in the mucous membrane of the frog intestine, disintegrative processes predominate over repair processes in the winter while the contrary is the case in the spring. In order to find whether the results obtained on mammals are general, or whether they apply only to the one class, it has been necessary to carry out similar experiments on representatives of other classes including the amphibia.

The aim of the present investigation has been to find whether those factors such as hunger, diet, and section of the sciatic nerve which have a decided effect in the case of mammals are also effective in the amphibia.

EXPERIMENTAL METHODS

The experiments were carried out on the crested newt (Triturus cristatus) in summer and winter. In the summer, the animals were caught in the reservoirs near Moscow shortly before the experiment was begun, while in winter the animals were used which had been kept for a considerable time in the laboratory. They were fed twice per week. Before the beginning of the experiment, the newts were starved for 5 days. Two experiments to find the effect of food, and one to find the effect of sciatic nerve section on the number of mitoses in the mucous membrane of the small intestine were carried out. The animals were killed by decapitation. The small intestine was removed intact. Part of the intestine (the caudal portion of the anterior half of the whole intestine) was fixed in Carnoy's solution. Paraffin sections 7μ thick were cut and stained in hematoxylineosin. A count was made of the different phases of the mitoses in the subepithelial outgrowths of the mucous membrane of the small intestine. The mitotic activity was expressed in each case as the number of mitoses per 1,000 cells.

The first experiment was carried out on 42 newts. Eight animals were killed in the fasting condition, and the remainder were fed at 10 a.m., after which 9 were killed after 2 hours, 8 after 6 hours, 9 after 10 hours, and 8 after 20 hours (from the time of feeding). In each animal 2,000 cells were examined.

EXPERIMENTAL RESULTS

It can be seen from Table 1 that 2 hours after feeding there is no difference in the number of mitoses between fasting and fed animals. Six hours after feeding there is an increase in the number of mitoses. This difference is statistically significant. The probability of such a difference arising by chance is less than 0.01. Ten hours after feeding the number of mitoses in the subepithelial outgrowths was reduced to the same level as in fasting animals, and was still the same after 20 hours.

TABLE 1

The Effect of Feeding on Mitotic Rate in the Mucous Membrane of the Small Intestine (First experiment)

No. of Mitoses Per 1,000 Cells				
In fasting newts	after feeding			
	after 2 hrs.	after 6 hrs.	after 20 hrs.	after 20 hrs.
20	12	17	30	24
15	8	19	7	8
9	21	22	10	18
13	14	21	27	14
20	19	31	12	21
21	18	14	6	5
11	12	29	23	11
27	20	32	15	12
	23		10	
On average 17	16.3	23.1	15.5	14.1

As the newts were killed at various times of day, the question arose as to whether the observed changes in the number of mitoses were due to diurnal variation and not to time after feeding.

To decide this question, we carried out an experiment in the winter on 24 newts. All the animals were killed at 4 p.m.: 9 – fasting, 8 – after 3 hours, and 7 – after 6 hours from the time of feeding. The last time interval was chosen because in the previous experiment an increase in the number of mitoses was observed 6 hours after feeding. The killing after 3 hours was carried out to determine an inner limit to the time for the increase in mitoses. In this experiment 4,000 cells were examined in each newt.

The results of the counts are shown in Table 2.

In the second experiment, the number of mitoses in the subepithelial outgrowth of the small intestine in fasting newts was 10.6 per 1,000 cells. After 3 hours, the number of mitoses showed a sharp increase to 23.1 per 1,000. After 6 hours, the number was 20.3 per 1,000.

There was no change in the relative numbers of the different phases.

The results of the second experiment show that the increase in the number of mitoses 3 and 6 hours after feeding are due to feeding, and are not the result of a diurnal variation of mitotic activity, because the newts were killed at the same time of day. Thus, in amphibia as in mammals, feeding causes an increase in the rate of mitosis in the mucous membrane of the intestine and the increases occur at the same time.

TABLE 2

The Effect of Feeding on the Mitotic Rate in the Mucous Membrane of the Small Intestine (Second experiment) (Number of mitoses per 1,000 cells)

In Fasting Newts	After feeding	
	after 3 hrs.	After 6 hrs
11	26	20
14	23	29
9	31	20
9	30	25
11	19	16
10	22	23
6	14	19
12	20	
14		
On average 10.6	23.1	20.3

TABLE 3

Effect of Sciatic Nerve Section on Mitotic Rate in the Mucous Membrane of the Small Intestine. Number of Mitoses per 1,000 Cells (Third experiment)

Control	After Section of Sciatic Nerve	
	after 40 min	after 3 hrs
7	21	29
19	19	12
11	25	12
19	22	25
16	21	20
7	25	18
7	9	
Average 12.2	20	19.3

The third experiment was carried out to find how mitotic activity is affected by stimuli unrelated to digestion. As stimulus we used sciatic nerve section, which is known to cause a considerable increase in mitoses in the epithelium of the crypts of Lieberkuhn in mammals.

The experiments were carried out in the summer on 20 newts, 7 of which were controls, and 13 of which had the sciatic nerve cut (6—3 hours, and 7—forty minutes before killing). All the animals were killed at the same time (at 4 pm). Two thousand cells were examined in each animal. The results are shown in Table 3.

It can be seen from Table 3 that a sharp increase in the number of mitoses occurs 40 minutes after section of the sciatic nerve, and this increase is statistically significant. Three hours after the nerve section there is still a significant increase.

The results of all the experiments failed to show any seasonal effect. This is perhaps because in winter the newts were kept in the warmth and were fed.

From these results we may conclude that the effect of adequate and inadequate stimuli on the mitotic activity of the epithelium of the crypts of Lieberkuhn in mammals, and on the homologous but structurally distinct subepithelial outgrowths in the amphibian intestine is substantially the same. The fact that in both amphibia and mammals, representatives of widely separated groups, adequate and inadequate stimuli increase the number of mitoses in the small intestine, suggests that the effect is also general for other species.

SUMMARY

The physiological regeneration of the epithelium of the mucous membrane of the small intestine was studied in newts in summer and in winter. The number of mitoses was increased in the subepithelial intestinal outgrowth in 3-6 hours after feeding. In 10 hours after the food intake the number of mitoses decreased to the level, which is characteristic of hungry animals. In 20 hours the mitotic activity was almost the same. The number of mitoses in the epithelium of the mucous membrane of the small intestine of newts shows pronounced increase in 40 minutes and in 3 hours after the resection of sciatic nerve. These data coincide with those which were found in the study of physiological regeneration of small intestine in mammals.

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THE EFFECT OF THE SYMPATHETIC SYSTEM AND ADRENALS ON SPERMATURIA IN THE FROG

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In 1947 Galli-Mainini [8] described a simple diagnostic test for pregnancy. The reaction consists of the effect on male frogs of hypophyseal or chorionic gonadotropin, contained in the urine of pregnant women; this induces a spermaturia which is readily detected by microscopic examination of a drop of urine taken from the cloaca.

Various investigations of this reaction have been made. V. P. Veis [3] found in frogs that after Sechenov inhibition, the spermaturia induced by injection of urine from pregnant females was considerably delayed. He supposed that the nervous center for the regulation of spermaturia was located in the spinal cord.

Galli-Mainini showed that in spinal frogs after removal of the brain, lungs, gut, spleen and hypophysis, the action of chorionic gonadotropin is preserved. He concluded that this reaction is due to the direct effect of the chorionic gonadotropin on the vesicula seminalis.

E. I. Kvater and I. F. Sokolova [5] consider that spermaturia in the frog is a reflex action. V. I. Babukhadiya [2] thinks that spermaturia may result from the direct action of the chorionic gonadotropin on the vesicula seminalis. Neither author gives any factual foundation for these views.

Since this reaction is so poorly understood, we decided to investigate the parts played by the sympathetic system and adrenals. Our starting point was the data of A. V. Tonkikh [7], who demonstrated the involvement of the sympathetic system in Sechenov inhibition, a result which was confirmed by I. V. Senkevich [6].

A. A. Atabek [1] induced spermaturia in the frog by injection of 0.3-1 ml of 1:1,000 adrenalin. We confirmed this in ten frogs; after injection of 1 ml of 1:1,000 adrenalin, spermaturia was obtained.

EXPERIMENTAL METHODS

Altogether 205 experiments were performed on lake and pond frogs (Rana ridibunda, R. esculenta).

In all frogs we first determined the rate and amount of spermaturia after injection of 3 ml of the urine from pregnant females into the dorsal lymph sac, and we recorded the room temperature. According to the number of spermatozoa in the urine, we were able to distinguish three degrees of spermaturia (the examination of the urine was carried out with the low power): reaction strongly positive – in each field of view more than 40-50 spermatozoa; positive reaction – more than 15-20 spermatozoa in a single field; weak positive reaction – occasional spermatozoa seen.

Three to four days after the strengths of these reactions had been determined, one of two operations was performed. At a definite time interval after the operation, the time of onset and the intensity of the reaction were again determined.

The first five experiments were designed to show the effect of Sechenov inhibition (Table 1).

TABLE 1

Sechenov Inhibition and the Spermaturia Reaction in Frogs

Experiment No. and Frog No.	No. of Frogs	Operation	Time Between operation and injection of Pregnant Urine	No. Frogs in whom Cut Brain Surface was not Stim. by Crystal of NaCl	No. of Frogs in whom Brain Surface was Stim. by Crystal of NaCl			Results of reaction	
					once for 1-2 min	once for 3 min	three times for 3 min at 25-min intervals	no. of frogs without delay in reaction	no. of frogs with delay in reaction
Exper. 1 1-34	34	Section through optic lobes and removal of brain anterior to this	After 24 hr	34	–	–	–	34	–
Exper. 2 35-42	8	Ditto	After 2 hr	8	–	–	–	8	–
Exper. 3 43-75	33	Ditto	After 24 hr	–	33	–	–	33	–
Exper. 4 76-95	20	Cut anterior to optic lobes through diencephalon and removal of brain anterior to this	After 1-2 hr	–	–	20	–	20	–
Exper. 5 96-115	20	Ditto	After 1-2 hr	–	–	–	20	4	16
Всего	115			42	33	20	20	99	16

EXPERIMENTAL RESULTS

Experiment 1. – 34 frogs; operation – section through optic lobes and removal of brain anterior to this. Injection of pregnant urine 24 hrs. after operation.

Results – rate of reaction the same before and after operation.

Experiment 2. – 8 frogs; same operation.

Pregnant urine injected two hrs after operation.

Results – rate of onset of reaction the same before and after operation.

Experiment 3. – 33 frogs; same operation.

Pregnant urine injected 24 hours after operation.

Immediately after the operation the cut surface of the brain was stimulated for 1 to 2 min with a crystal of sodium chloride.

Results – rate of onset of the reaction before and after operation the same.

Experiment 4. – 20 frogs; operation – section anterior to the optic lobes through the diencephalon and removal of parts anterior to cut.

Pregnant urine injected 1-2hrs after operation. Immediately after the injection the cut brain surface was stimulated for 3 min. with a crystal of sodium chloride.

Results – Rate of onset the same before and after operation.

Experiment 5. – 20 frogs; operation – section through diencephalon anterior to optic lobes and removal of brain anterior to cut.

Pregnant urine injected 1-2 hours after operation with simultaneous stimulation of cut surface of brain with crystal of sodium chloride 3 times for 3 min at intervals of 25 mins. (These experiments were carried out in the same way as by V. P. Veis.) The pregnant urine was injected after the first stimulation.

Results – in 4 frogs the rate of onset of the reaction was the same as before operation; in 16 frogs the reaction was delayed from 30 min to 1 hr, i. e., we confirmed the results of V. P. Veis.

Experiments 3 and 4 showed that, as regards Sechenov inhibition, a single stimulation of the cut brain surface does not change the time of onset of the reaction. Sechenov inhibition of the spermaturia reaction occurs only on stimulating the cut brain surface three times for 3 min at 25-min intervals with a crystal of sodium chloride; out of the 20 frogs of experiment 5, in 16 there was a delay of 30-60 minutes. This indicates a reflex regulation of the spermaturia reaction. We may suppose that there are nerve centers in the cord which regulate this reaction, and that their suppression in the Sechenov inhibition experiment causes a delay in the spermaturia reaction.

In 24-48 hr observations on frogs operated by Sechenov's method, we found spontaneous spermaturia to occur in 25 animals. This took place 2-24 and even 48 hrs after the operation and was observed most frequently in summer and winter frogs. In our view, the spontaneous spermaturia is due to the release of the centers in the cord from the inhibitory influence of the higher nervous centers.

Sixty-five experiments were performed to investigate the reaction of the sympathetic and adrenal system.

The adrenal glands were cauterized with an electric thermo-cautery, using A. V. Kibyakov's method. On the fifth day after the operation the rate of onset and the strength of the reaction to the injection of pregnant urine was determined. According to A. V. Kibyakov [4] and his co-workers, it is at this time that the sympathetic adrenal system is at its lowest functional level.

The results of the experiment are shown in Table 2.

TABLE 2

The Sympathetico-Adrenal System and the Spermaturia Reaction

Experiment No.	No. of frogs	Type of operation	Time between experiment and operation	Results of the spermaturia reaction		
				No. of frogs in which the rate and strength of the reaction were the same before and after operation	No. of frogs in which there was no delay of onset, but a reduced intensity	No. of frogs with delayed and reduced reaction
141-205	65	Cauterization of adrenal glands with electric thermo-cautery	On the fifth day	16	8	41

In 41 of the 65 frogs, the spermaturia was reduced in amount and there was a delay of from 40 min to 2 hrs. The delay was most clearly shown in the spring and summer frogs.

In 8 animals the reaction was less in amount, but the time of onset was the same.

In 16 frogs the rate and amount were the same before and after operation.

Thus, in most frogs removal of the adrenal glands (essentially the adrenal medulla) had an effect on the spermaturia reaction. The absence of this effect in some of the frogs may be due to incomplete cauterization of the medulla of the gland, or to the presence of additional chromaffin tissue.

Our experiments showed that the sympathetico-adrenal system is concerned in the regulation of the spermaturia reaction.

The physiological mechanism of this reaction is therefore basically reflex, and includes centers in the diencephalon and cord, as well as the sympathetico-adrenal system.

SUMMARY

205 experiments were conducted to elucidate the role of Sechenov's inhibition and the sympathetico-adrenal system in the mechanism of spermaturia. This reaction was stimulated by urine of pregnant women.

It was established that the physiological mechanism of spermaturia in frogs is of a reflex nature. It includes the transitional and spinal centers and those of the sympathetico-adrenal system.

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• In Russian.

CHANGES IN THE GENITAL TRACT DURING ESTRUS

COMMUNICATION III. THE EFFECT OF INSEMINATION

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A considerable amount of indirect evidence has been published to the effect that insemination is a powerful stimulus to the female sexual system. Sperm has been shown to have an effect on isolated organs and tissue [7, 9], biologically active substances such as the enzymes hyaluronidase and mucinase [5] have been found in it, and the effect of insemination on female sexual function has been demonstrated [4, 8]. It must, however, be pointed out that there is no direct evidence of the response of the female genital tract to insemination. The difficulty is that in the uterus and vagina during estrus, i. e., at the time when insemination normally occurs, destructive changes occur in the mucous membrane and these alternate with periods of proliferative repair in the postestrus periods. For mice and rats [6, 10, 3], these histological changes have been described broadly as follows. During the proliferative phase, there is an increased rate of cell division of the epithelium of the genital tract. The epithelium of the vagina becomes reduced from two layers to one, and its outer part becomes cuticularized. There is a hypertrophy of the mucous membrane of the uterus, and the epithelial cells become regularly arranged side by side. The mucous membrane secretes actively, the stroma is loose, and the uterine glands have a large lumen. During the destructive phase there is a mass infiltration of leukocytes into the wall of the tract, and many enter the lumen. The superficial layer of the vaginal epithelium comes away. The uterine epithelium becomes uneven, the outlines of the cells and the basement membrane become less distinct, vacuoles, granules, etc. appear in the lumen, and villus-like outgrowths are formed from which cells break off, often together with the leukocytes which have penetrated into them. Until now, the biological significance of these changes has not been sufficiently well understood. We suppose that they constitute the morphological basis of the processes occurring at the same time in the lumen of the genital tract. The occurrence of the proliferative phase facilitates the reception of the ejaculate, the activation of the sperm [1], and its movement along the tract. After fertilization, an active destruction of the contents of the tract occurs, and the vaginal plug and ejaculate are voided to the outside [2]. These processes are brought about by destructive infiltration of the vaginal and uterine walls. This destruction is a preparation for the reformation of the mucous membrane and its subsequent proliferation. The latter comes to constitute a new proliferative phase in cases where the cycle is not completed by fertilization; when this does occur, the proliferative changes represent an adaption to implantation. Thus, all these changes in the wall of the genital tract, which we have described by the term "estrus complex", constitute an essential part of the sexual cycle of the female and play an important part in the reproductive process. On account of these changes, and in particular on account of the destructive infiltration, it is not possible to elicit reactive changes in the uterus and vagina in response to insemination, or to find the nature of any such changes.

In order to overcome this difficulty, we used females which were serviced outside estrus and after ovariectomy. The mucous membrane of the genital tract of these animals was in the resting condition, and reactive changes in it could be easily demonstrated. Five experiments were carried out. The animals were killed 7-10 hours after being serviced; the ovaries, vagina, and uterus were fixed in Bouin's fluid or in Zenker-formol, and embedded in

paraffin. Cross sections and longitudinal sections of the organs were stained in hemalumeosin. The sexual organs of 26 virgin females were taken as controls, of which 20 were normal (in different stages of the sexual cycle), and 6 were ovariectomized.

In the 1st experiment, 8 females were serviced at an early stage of proestrus. In one case, histological examination showed a massive infiltration of the uterine stroma and epithelium with polymorphonuclear leukocytes, many of which were found in the lumen; there was an uneven distribution of the nuclei of the epithelium, a formation of villi which shed cells from their apices into the lumen, a disappearance of the basement membrane etc. i. e. there was a destructive infiltration such as normally occurs in the second half of estrus after ovulation (ovulation did not occur in any animals in the 1st experiment). In 6 animals all these phenomena were weakly shown, and in one case were not shown at all (i. e. the uterine mucosa of this female was not in any way different from that of an unserviced female at the proestrus stage).

In the second experiment 11 females were serviced in the resting stage. Copulation caused destructive infiltrational changes in the uterine mucosa; these changes resembled those in the first experiment, but were very clearly shown in 8 out of the 11 animals.

In this experiment the formation of epithelial villi and the shedding of cells into the lumen was not well shown.

In the 3rd experiment, in 7 females serviced after estrus, destructive infiltration in the uterus was well shown in all animals. This may be partly due to the fact that after estrus the normal destructive phase is terminating. We may note, however, that copulation delays its completion.

In the 4th experiment, in females serviced 7-10 days after ovariectomy, the uterine reaction to insemination was very similar to that of experimental animals (Fig. 1).

The principle uterine reaction was shown in experiment 5, in females serviced 18 or more days after ovariectomy. In all the previous experiments, the changes in the mucosa were approximately uniform over the whole organ, but in this series the picture was very varied. Some parts remained intact, while others were more like the mucosa of females in the 2nd or 4th experiments, in which the reaction was very marked. In these places there had been a desquamation of the epithelium over a considerable expanse, the sperm had penetrated in a broad stream into the stroma and had infiltrated it right up to the muscle layer, the place where penetration had occurred being surrounded by a thick band of leukocytes, i. e., there had been a typical inflammatory reaction.

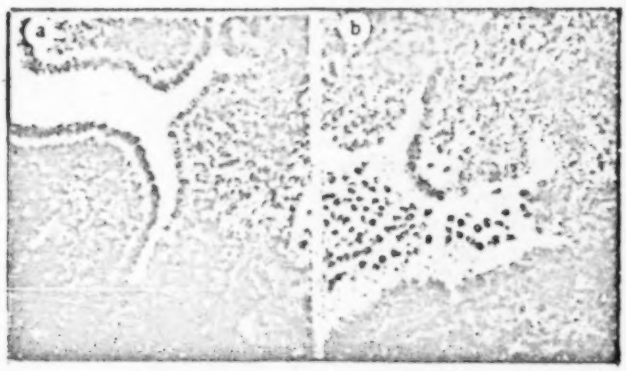

Fig. 1. Changes in the genital tract of the rat: a) Uterine wall of ovariectomized female. Even layer of short epithelium; b) the same 10 hours after copulation. Disorderly arrangement of the epithelial cells, desquamating in parts into the lumen. Leukocyte infiltration of the mucosa. Large number of leukocytes in uterine canal.

Investigation of the vagina in ovariectomized animals showed that here the stimulation of natural insemination, i. e., chiefly that of the vaginal plug, results in a considerable destructive infiltration (Fig. 2). It must be noted that all the above changes in the female genital tract do not occur if copulation is not completed, i. e., if there is no ejaculation and entry of sperm into the uterus. In order to find the effect of chronic stimulation, we also carried out experiments on 3 mice with copulation repeated at intervals of 4-5 days. Histological examination of the sexual organs of these females showed that the uterine wall had hypertrophied as compared with that of the control animals, the mucosa was thicker, and the epithelium better developed and richer in cells. The destructive infiltration following repeated insemination was more reminiscent of the reaction of ovariectomized females (absence of pathological signs and occurrence of the normal destructive phase of estrus, as for example the formation of epithelial villi, etc.).

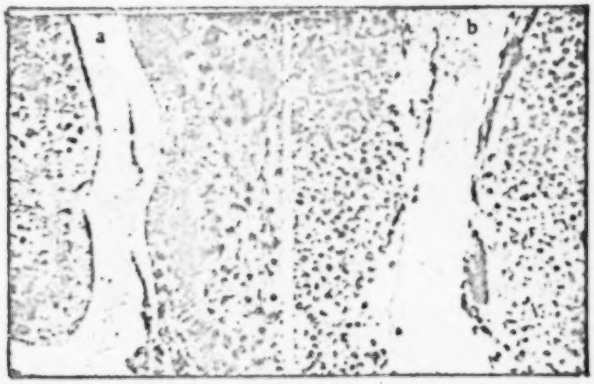

Fig. 2. a) Vaginal wall of ovariectomized female; b) the same, a few hours after copulation. Marked infiltrational and destructive changes.

Thus, our experiments show that natural insemination is a powerful physiological stimulus, which elicits destructive infiltrational changes in the genital tract of the female similar to those of the corresponding estrus phase. This phase, as had already been said, occurs in the second half of estrus and in the postestrus period, i.e., it occurs a few hours after natural insemination (which usually takes place in proestrus or at the beginning of estrus) and directly after ovulation, and therefore after fertilization.

The physiological basis of the normal estrus cycle is the complex neurohumoral mechanism of the sexual cycle. However, the results of the experiments here show that a very important and, biologically speaking, decisive part in eliciting the destructive phase in particular, is that of the powerful external stimulus of the act of insemination. Finally, the destructive changes obtained on inseminating nonestrus and ovariectomized females are not completely analogous to those of the destructive phase of normal estrus, though the differences are explicable in terms of the specific condition of the animals and of their reproductive organs. These differences are proportional to the degree of involution of the female genital system, and in animals which have been ovariectomized for a considerable time the reaction shows no qualitative differences. The act of insemination is no less important for the realization of the phase of proliferative repair. This is shown by the experiments with repeated copulation. It is generally accepted that the processes of destructive infiltration and proliferative repair are intimately associated.

Thus, natural insemination, considered as a biological stimulus, plays an important part in the realization of that complex cycle of changes in the uterus and vagina included in the term "estrus complex." We may suppose further that this complex arose originally as a reaction to insemination. In the course of phylogenesis, this reaction became separated from the original external stimulus through the formation of associated reflex pathways. Consequently, a self-contained neurohumoral estrus mechanism evolved, as a result of which the cyclic change which composed it can now take place without insemination occurring.

SUMMARY

The reaction of the genital tract of female mice in various stages of the estrus cycle and of ovariectomized females to coitus was investigated. Discharge of semen caused destructive- infiltrative changes in the uterus and vagina, which resembled the physiological destruction of the mucous membrane of these organs which was observed in the second half of estrus.

Similarity was the greatest in females in cases of copulation at the stage of rest or soon after ovariectomy (7-10 days).

Coitus plays an important part in the process of formation and maintenance of the cyclic destructive and proliferative changes of the mucous membrane of the genital tract of female mammals.

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* In Russian.

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MORPHOLOGY AND PATHOMORPHOLOGY

EXPERIMENTAL RENAL INFARCTS

COMMUNICATION II. ARTERIAL INFARCTS

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A great deal of evidence has been published which shows the great importance of the tone of the coronary vessels and their collaterals in the etiology of myocardial infarct, which makes it necessary to reconsider Konheim's theory of infarction. A. V. Lebedinsky, V. I. Medvedev, and I. A. Peimer [5] showed that a spastic condition develops in the right coronary artery following ligature of the left.

These results have been confirmed for the kidney. It has been shown that ligature of the pelvic branch of the anterior renal artery induces reflex spasm in the corresponding branch of the posterior renal artery [6, 7].

These results give reason to suppose that the reflex vasomotor changes may play an important part in the etiology of infarction. However the question of the effect of the nerve supply to the organ on infarction has not been sufficiently studied. In the present work, we report the results of an investigation into the effect of renal denervation on infarction.

EXPERIMENTAL METHODS

The experiments were carried out on 97 white rats in whom renal infarcts were induced by ligature of the pelvic branch of the posterior renal artery. An extracelomic lumbar approach to the kidney was used. In the first set of experiments, with 43 animals, the development of infarction was studied while the nerve supply to the kidney was intact. In the second set of experiments, with 32 animals, the left kidney was denervated 7-12 days prior to ligature of the vessel. For the denervation, the entering blood vessels and the upper quarter of the ureter were freed from surrounding tissue, and all nerves entering the kidney visible to the naked eye were cut. The renal vein was separated from the renal artery. Where the latter left the aorta, the arterial sheath was removed, and with it the superior and inferior renal ganglia. After this the adventitia of the above two vessels and the upper part of the ureter were treated with a 2% solution of phenol, after which the parts were washed with saline. After this, the capsule of the kidney was removed. In the third group of experiments, on 22 animals, the fibrous capsule of the kidney was removed either at the same time as, or 8-12 days before ligature of the artery. The animals were killed 40 minutes, $1^1/_2$, 3, 6, 12 and 24 hours, and 3, 7, 14, 21, and 30 days after the ligature had been made. The material was fixed in 8-10% formalin and embedded in celloidin, or else sections were cut with a freezing microtome. The sections were stained in van Gieson's hematoxylin-eosin, fuchselin for elastic fibers, with Sudan III for fat and by Goldman's method for leukocytes. In some of the animals of groups 1-3, angiograms were made for the study of the blood supply to the kidney, the vessels being perfused with a mixture of lead acetate in 3% gelatin [2]. The whole kidney was removed, and placed with the hilus upwards; it was then cut into two parts the division following the distribution of the pelvic artery. In some of the experiments stereoroentgenograms were made. The pictures were examined with a magnification of twenty times, as well as using a stereoscope.

At an early stage of the development of the infarct the morphological changes and the angiograms were the same in all the groups of experiments. In the first 12 hours after the ligature a necrotic area developed, surrounded by a hemorrhagic rim. At the beginning of this period there was a granular and watery dystrophy which was most clearly shown in the convoluted tubules, some of which perished after only 3 hours. The cells of the other regions of the tubules were preserved at first somewhat better, and the greatest resistance to the hypoxia was shown by the endothelial capillaries of the glomeruli. The angiograms showed that in the early stages the arteries in the region of the infarct are incompletely filled with the lead acetate fluid (Fig. 1, b). Under the microscope it could be seen only in some of the small blood vessels.

From the angiographs it could be seen that in both control and experimental animals there are comparatively few anastomoses between the small internal branches of the renal artery (Fig. 1).

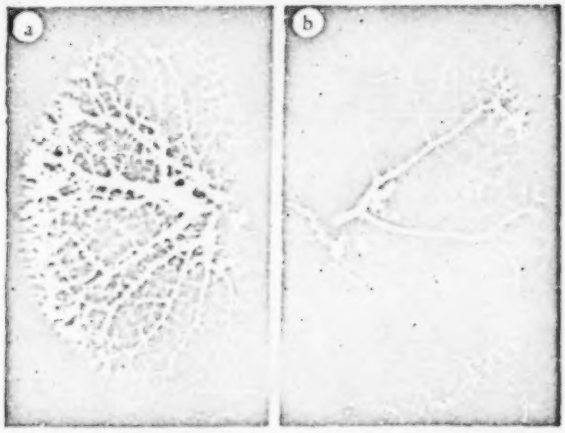

Fig. 1. Angiogram. a) Right (healthy) kidney; b) left denervated kidney. In the region of the infarct the arteries have not been filled with the contrasting substance. Anastomoses between the vessels can be seen. Length of experiment 24 hours. Enlargement on printing $3^1/_2$ times.

On account of the paucity of collaterals [6, 7], the denervation, which prevents the development of spasticity in the anterior pelvic artery from occurring after ligature of the pelvic branch of the posterior renal artery, and which therefore increases the blood supply to the ischemic zone, had comparatively little effect on the development of the infarct. For the same reason, on account of the limited potential development of the extrarenal collaterals, their removal with the capsule of the kidney had no great effect on the process of infarction.

At later stages, definite differences were shown between the innervated and denervated kidneys. Investigation of the infarct in the denervated kidney showed: (1) an increased inflammatory reaction, with exudate; the band of leukocytes around the edge of the infarct was broader and contained a larger number of leukocytes (Fig. 2, a and b); (2) there was an increased amount of new connective tissue around and in the infarct; (3) the process of transformation of the granulation into fibrous tissue to form a capsule around the infarct with layers penetrating into it proceeds more rapidly, being approximately one week in advance of the corresponding process in the control animals.

Similar but less well-marked changes were shown in the experiments with decapsulated kidneys.

We have found no publications describing the effect of denervation and decapsulation of the kidney on the development of the inflammatory reaction round an infarct. However, our findings confirm those of D. E. Alpern [1], V. G. Eliseev [3], and other workers who found an increased inflammatory reaction following section of the sympathetic nerves or denervation of the tissue, and who also found an increased permeability of the blood vessels

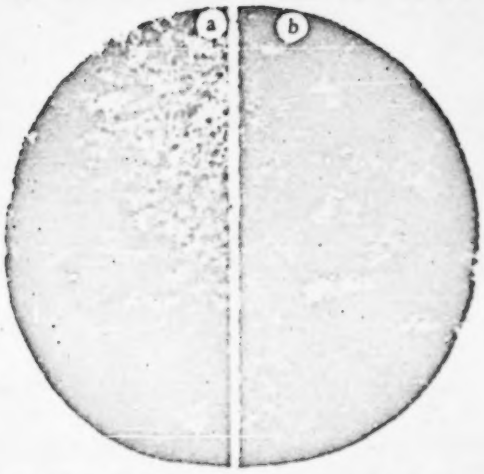

Fig. 2. Band of leukocytes around periphery of infarct in experiment lasting 24 hours.
a) Innervation intact; b) denervated kidney, stain hematoxylin-eosin, Magnification, eye piece 10 objective 20.

after denervation. B. I. Lavrentyev [4] showed that the nervous system regulates the degree of cellular differentiation. We may suppose, therefore, that denervation may increase proliferation of connective tissue, as we in fact observed in our experiments.

Decapsulation itself, which at the present time is considered by many workers to be a partial denervation, had a similar but less strong influence on the reactive inflammatory process and the organization of the infarct.

SUMMARY

The development of infarction after ligature of the posterior pelvic artery of a denervated and decapsulated kidney and of a normal kidney with intact innervation was compared. No difference in the development of infarctions in both kidneys could be noted at an early stage, but later on reactive inflammation, growth of connective tissue and cicatrization were enhanced in the denervated kidney.

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METHODS

A RAPID METHOD OF SCANNING ELECTROPHOROGRAMS

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Paper electrophoresis for separating serum proteins has become widely used. It is very time-consuming to the electrophorograms by eluting the dye from the cut-up paper strip. Densitometers have been described for rapid electrophorogram scanning, but they are at present difficult to obtain.

We used an EKP-4m electrocardiograph, modified as little as possible so it could still be used for its main job, to scan the electrophorograms. The additional devices could be partially produced from apparatus to hand.

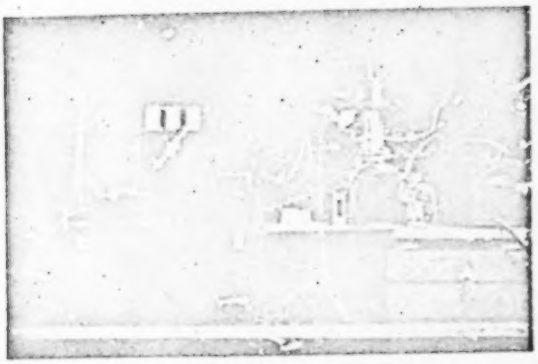

Fig. 1. A general view of the EKP-4m electrocardiograph plus the additional devices.
1) Photomultiplier or photocell; 2) light source; 3) control arc; 4) spool carrier with the electrophorogram strip mounted on it; 5) tension roller; 6) drive roller (drive from a synchronous motor plus reduction gear); 7) plate supply; 8) stepdown transformer; 9) stand.

Flynn and Mayo's [2] and Gurvich's [1] methods were used for serum protein electrophoresis. The strips were prepared from filter paper.

An advantage of this method is that it is relatively simple. No sensitive reflecting galvanometers (electrometers) requiring special locations are used, so the apparatus can be rapidly moved about and set up.

Figure 1 gives a general view of the electrocardiograph plus additional devices.

When the additional devices are being set up care must be taken not to use a beam over 0.5 mm in width and length 3-4 mm less than that of the strip from the light-source.

The photocell current is proportional to the light intensity. When the strip is drawn between the light source and photocell a light beam of variable intensity is produced. The photocell current changes are amplified and transmitted to the electrocardiograph galvanometer and recorded on the film.

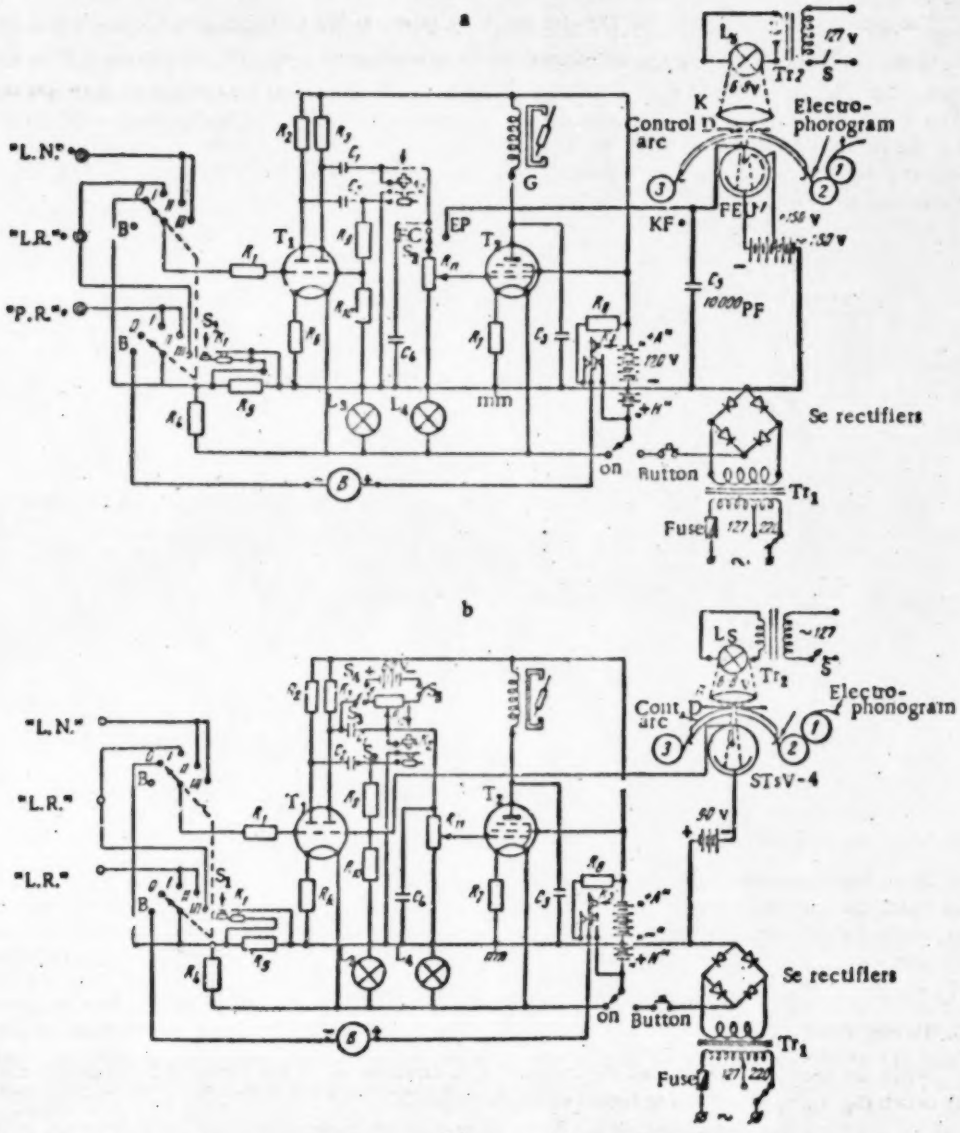

Fig. 2. Basic circuit of the EKP-4m adapted for scanning electrophorograms: First and second variants (a and b respectively). 1) Drive roller; 2) tension roller; 3) spool carrier with the strip mounted on it; L_5 light source, K condenser lens, D diaphragm. The other parts are explained in the text.

The electrocardiograph had all the units required for recording the photocell current. We tested two variants, one with a FEU-1 photomultiplier and the other with a STsV-4 photocell.

* Transliterated from Russian. No explanation given in text.

In the first the Switch S_2 (Fig. 2a) was fitted to the top panel of the EKP-4m; this enabled one to connect the top end of R_{11} either as normally in the EKP-4m or to the positive side on the FEU-1. In the latter case R_{11} is the photocell load, which supplies the voltage to be amplified to the grid of the amplifier tube T_2 (SO-244). A 10,000 μF capacitor was connected across R_{11} to eliminate 50 cycle line-frequency interference and pulsations due to the low thermal inertia of the lamp filament.

The plate battery voltage of the EKP-4m had to be raised to 120 v.

In the second variant two stages of the EKP-4m amplifier had to be used. The positive side of the STsV-4 was connected to the right-hand grid in T_1(SO-243). A BAS-60 battery was installed in the EKP-4m with a 0.3 MΩ variable resistor (R_{12}) together with a multibank switch to cut in this circuit, which is more complex than that of the first variant (Fig. 2b). This multibank switch does the following operations: cuts out C_2 (S_2), cuts in the 0.3 MΩ variable resistance (R_{12}(in parallel with C_1(S_3), and connects the BAS-60 battery to R_{12} (S_4 and S_5). The check switch must be set at 0 and the gain switch on the 10th gain position, i. e., hard right.

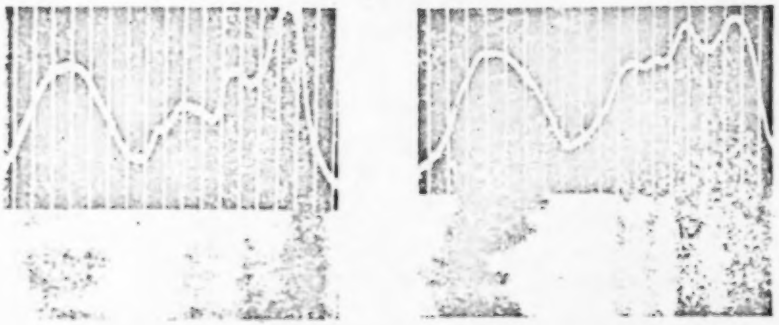

Fig. 3. Electrophorograms from serum proteins of two rabbits with different immunities.
The curves taken on the EKP-4m are shown at the top.

When electrophorograms are being scanned the end of one is fixed to the beginning of the next. A strip comprised of 15-20 such electrophorograms is then wound into a spool (Fig. 1, 4). The front end of the strip (which is an extra piece of paper, and not the first electrophorogram) is inserted into the control arc and gripped between the drive (6) and tension (5) rollers; the apparatus and light source (2) are then switched on.

When the first variant is used the indicator spot on the EKP-4m should be adjusted to the lower part of its range (using the corrector) with a white section of the first electrophorogram under the light source; then the most deeply stained part is placed under the source, and the indicator spot should pass to the other extreme position. The indicator spot can be set to this position by adjusting the light source intensity (rheostat, transformer). This operation should be repeated twice.

Having thus set the white and blue levels the recording will occupy the full width of the film.

When the second variant is used the indicator spot should be set at the center of its range and the multibank switch (S_2, S_3, S_4 and S_5) set to record electrophorograms.

Since the indicator spot does then not appear in its former place it should be returned to the proper position by adjusting R_{12}. The white level will then be at the top of the scale and the blue at the bottom. The white and blue levels are then set.

Then, in both variants, the film-drive mechanism in the electrocardiograph is switched on as well as the synchronous motor (6) which drives the electrophorograms. When all the electrophorograms have passed before the photomultiplier (1) the electrocardiograph and synchronous motor are switched off and the film removed from the cassette and developed.

With the developed image mounted on transparent squared paper the area corresponding to each protein fraction is determined and the percentage relations calculated (see Fig. 3). The total amount of protein being known (from refractometry) the absolute protein fraction contents in the serum can be determined when the time variations of the protein fractions are being studied in a given case.

Chromatograms etc. can be scanned in the same way.

Thus this method amounts to counting the areas of the protein fractions, since the time taken to get the graphic record is practically the same whether one or many electrophorograms are made up into the strip.

The results obtained with the electrocardiograph were checked by eluting the dye and photometering the solutions on an FEK-M, the following results (Table) being obtained.

Method	Type of Measur.	Albu-men	α-Glo-bulin	β-Glo-bulin	γ-Glo-bulin
Elution	Area	83	39	33	45
	%	41.5	19.5	16.5	22.5
	Optical density	0.366	0.152	0.155	0.224
	%	40.8	17.0	17.3	24.9
EKP-4m	Area	322	146	108	140
	%	45	20.4	15.1	19.5
Elution	Optical density	0.4	0.18	0.109	0.141
	%	47.8	21.7	13.3	17.2

The data show that the maximum discrepancy was 2.5-2.8%, and we cannot for certain say which method was responsible for these small differences.

Using a PS-381 exit collimator we established the concentration limit beyond which the linear ratio between the dye adsorbed by the protein and light absorption was not obeyed. The linear ratio held up to 2 mg protein per cm^2 of paper. So we used lesser amounts (less than 0.5 mg per cm^2), so there was no danger of the Beer-Lambert law not being obeyed. But the method can also be used with large amounts of protein. It was shown that the extinction and protein concentration were linearly related up to 128 mg/cm^2. In this case the record should be projected onto semilog paper using a magnifier.

With our method the relation between the protein fractions can be determined quantitatively within 5-6 hours of taking the blood for a large series of serum specimens, since only three hours is required to get a good separation of the protein fractions on the paper under our conditions.

Since electrocardiographs are widely used in most medical and scientific institutions this method could find extensive application in clinical practice.

SUMMARY

Two methods of preparing electrophorograms with the electrocardiograph EKP-4m are described. They depend on the type of apparatus at hand, the photomultiplier FEU-1, or the photocell – STsV-4. Additional devices can be mounted from common laboratory equipment. Results are comparable with electrophorograms prepared by other methods, but the rapidity of preparing whole series of electrophorograms with unbroken graphic reproduction makes this method preferable. If protein concentrations of more than 2 mg/cm^2 are used the record should be projected with a photomagnifier on semilog paper.

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THE USE OF ESSENTIAL OILS IN HISTOLOGIC PRACTICE

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A series of essential oils such as oil of cloves, bergamot, camphor, and others are used in various basic histological procedures principally for the purpose of extracting the celloidin and clearing the sections. This is especially important before many histologic and bacteriologic stains can be used.

Cajuput terpene oil and oil of cloves, which are so widely used for these purposes, are expensive and difficult to obtain.

In our search for substitutes we employed for experimental histologic procedures the essential oils of the following plants: lemon sorghum (Cymbopogon citratus), eugenol basilik (Ocimum gratissimum), lemon eucalyptus (Eucalyptus citriodare), eucalyptus (Eucalyptus viminalis), pink geranium (Pelargonium roseum), patchouli (Pogostemon patchouli) and melalevka (Melaleuca alternifolia).*

EXPERIMENTAL METHODS

Extractions from celloidin sections were conducted in the following manner. The section is removed from the water on to a glass slide, smoothed, and dried with a piece of filter paper. After the celloidin is extracted from the slice, the glass slide is rinsed out first with absolute, and then with 96% alcohol. Then it is rinsed with water and stained according to various methods. Celloidin can also be removed by dehydrating the section in 96% alcohol and then adding it to a jar with the essential oil. After rinsing with alcohols and water, we can then stain these sections by the same procedures employed on the frozen slices.

After removing the celloidin, we stained the sections with hematoxylin-eosin, picrofuchsin and Mallory. The sections subjected to the action of the essential oils retained their capacity to stain, the color being retained for as long as $2^1/_2$ months.

Celloidin was entirely removed within 7 to 15 minutes by the oils of lemon sorghum, eugenol basilik, lemon eucalyptus and pink geranium, the sections clearing at the same time. Celloidin was removed most rapidly by oil of pink geranium (7 minutes).

Also investigated was the possibility of using various volatile oils in staining nerve cells. To test this, material from monkey brains was taken, fixed in 96% ethyl alcohol, and imbedded in celloidin, as in common procedures for staining nerve tissues. We used 0.1% toluidine blue in aqueous solution followed by rinsing in several portions of 96% alcohol. Instead of clearing the slices with the usual cajuput oil, we used oils of melalevka, eugenol basilik, patchouli, pink geranium and lemon eucalyptus. As controls, a portion of the sections was differentiated with cajuput oil. Later, as with clearance with cajuput oil, the essential oils were washed out with pure xylene and the sections were covered with Canadian balsam.

* These oils were obtained from the Sukhumi essential oils zonal station where their properties were first studied in detail by the director of the biochemical laboratory of the station, A. A. Pravdolyubov, and then turned over to us for further experimentation.

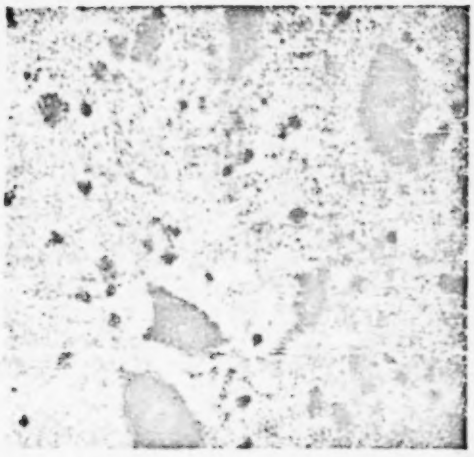

Fig. 1. Staining of the nerve cells from spinal cord anterior horn with toluidine blue. Cleared with patchouli oil. Objective 40, ocular 7.

Fig. 2. Pulmonary tissue stained after van Gieson. Celloidin extracted with lemon sorghum oil. Objective 20, ocular 7.

EXPERIMENTAL RESULTS

The nerve cells and especially their chromatophilic substances stained best after the sections had been cleared with melalevka and patchouli (Figs. 1 and 2). Both sections were indistinguishable from the controls. The chromatophilic substance stained intense blue with a lilac sheen. The nuclei of the nerve cells, with the exception of the nucleoli and the fibrous brain structures, remained unstained. The glia cells were stained in the usual manner. Oil of melalevka was superior to patchouli oil in that the sections cleared within 1-2 minutes, as against 7-10 minutes for patchouli.

The investigations with the other essential oils gave less favorable results. When oils from eugenol basilik, pink geranium and lemon eucalyptus were used, clearing occurred within 7 to 15 minutes. However, only a portion of the nerve cells resembled the control sections in acquiring the blue-lilac sheen. In a number of the cells, the chromatophilic substance, as well as the protoplasm and some nucleoli, stained green. At the same time the contours of some of the cell elements remained indistinct. The entire slice stained diffusely a light green tone. This staining defect was most pronounced with eugenol basilik oil. When the sections were treated with pink geranium, only individual nerve cells acquired the green shade. In sections with lemon eucalyptus, the nerve elements stained well and only occasional tissue and glial cells took on the green color. When the last three essential oils were used, the celloidin was either partially or fully dissolved at the moment of full clearing, depending on the thickness of the section.

It should be observed that all the volatile oils studied by us are readily removable with ethyl alcohol, but the pink geranium and eugenol basilik oils could not always be fully washed out with xylene. The possibility is not excluded that this reflects on the quality of the stain after the preparation is stored.

Thus, instead of using oil of cloves for the extraction of celloidin from the preparations, we found that the following volatile oils could be used without lowering the quality of the sections: eucalyptus (Eucalyptus citriodare), lemon sorghum (Cymbopogon citratus), eugenol basilik (Ocimum gratissimum) and pink geranium (Pelargonium roseum).

The essential oils of melalevka and patchouli appear to be the best substitutes for cajuput oil in the staining of nerve cells.

SUMMARY

Clearing of microscopic sections as well as extraction of celloidin from them can be successfully accomplished with the use of such essential oils as Cymbopogon citratus, Ocimum gratissimum, Eucalyptus citriodare, and Pelargonium roseum instead of the usual oil of cloves. For differentiating and staining nerve cell preparations, good results have been obtained with the use of the essential oils of Melaleuca alternifolia and Pogostemon patchouli instead of cajuput terpene oil.

A METHOD OF MICROSCOPIC STUDY OF THE LIVING PERIPHERAL BLOOD CIRCULATION IN MICE

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It is well known that in the study of the physiology and pathology of the local circulation so-called "transparent cameras" are used. This method was first developed by Sandison [1] and perfected later by Clark and coworkers [2], while other investigators, particularly Williams [3], apart from students using it only in animal ears, adapted it for use in a portion of rabbit skin. All the cameras proposed by the various authors may be divided into two types. One is so constructed that the slit of the camera is built of a transparent material so that the tissues of the organ into which it has been inserted will grow into the visible field and so become the object of study; it follows that this camera type permits observation of regenerating tissues. The construction of the other camera type presupposes inclusion within the slit of the transparent camera of already formed tissues. To this second type of "transparent cameras" may be added the camera recently described by Algire [4] as being constructed specifically for insertion into skin folds of laboratory mice. This Alger Modification is of great interest as the mouse is a readily accessible animal, easily immobilized and quite conveniently adapted for microscopic observations.

However, the Algire camera has very serious defects: it is complicated, has many detailed parts, and has the especial drawback that its introduction into a skin fold is attended by marked trauma of the tissues and marked circulatory disturbance. This last effect is associated with the need for numerous sutures and holes in the skin for the passage of bolts which stretch the skin as a result of excessively high rim edges. All this led us to propose a modification of the "transparent camera" that avoided the defects just mentioned.

The camera consists of a zinc frame with a cover glass glued to it and having two assisting foundation bars (Fig 1). The dimensions as given in Fig. 1 are calculated for a mouse weighing 22-23 g; they may be altered to fit the size of the animal. The cover glass is given an edge to correspond to the frame dimensions and is glued to it with BF-2 glue.

The camera is introduced operatively under aseptic conditions; it can be done with use of narcosis (chloral hydrate solution 0.0003 g per g of animal) or without it.

The mouse is immobilized on a special table (which may be replaced by the camera plate) having five openings whose positions correspond to the paws and the middle of the tail when the mouse is prone on the table. Through each of these openings is passed a piece of doubled-up rubber tubing, or a rubber cord. The paws and tail of the mouse are inserted into the corresponding rubber loops, the free ends of which are drawn tightly to the undersurface of the table so that the mouse is firmly attached in a prone position to the table. The immobilized animal is covered with gauze sponges. Then the skin of the animal is depilated with the aid of taro starch in

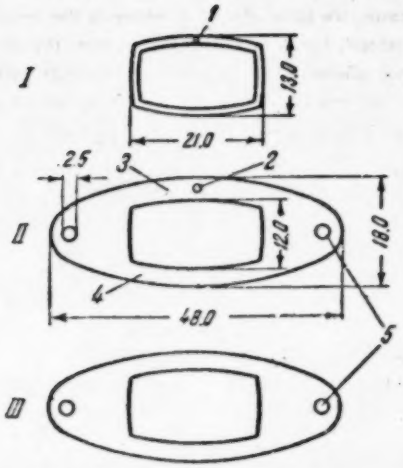

Fig. 1. I) Zinc frame to which is glued a cover glass, II) basic foundation bar, III) additional foundation resting bar. All dimensions in mm.

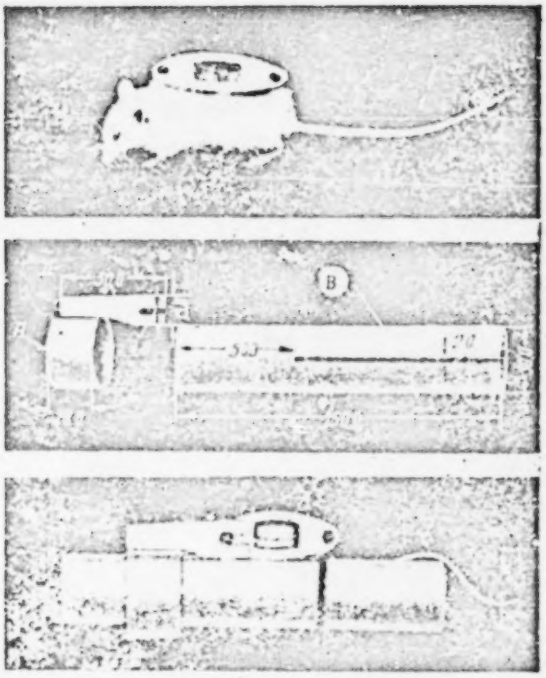

Fig. 2. I) Mouse with introduced camera, II) dis-assembled fixator. A) ring with fork; B) tube. III) mouse with camera inserted into fixator.

such fashion that there remains along the course of the backbone a narrow strip of hairs to which is glued (with BF-2 glue) the upper edge of one of the foundation bars (Fig. I, 3). After a few minutes, when the glue has dried, the assistant grasps with a Pean clamp from the right side this foundation bar and places it, with the attached skin fold in a vertical position. With an eye scalpel, in the outer wall of the depilated skin, a square opening is made in which the scalpel is introduced and the fascia of the intact skin are then carefully removed. We recommend irrigation of the internal layer of the depilated skin with a penicillin solution. Then, through the aperture in the skin we introduce the zinc frame with the glued on cover glass doing this in such a manner that that side of the frame to which the glass is glued faces the intact wall of the depilated skin. The zinc frame is fixed to the foundation bar with a stitch passed throught the upper side of the frame (1) and also the center of the upper edge of the foundation bar (2). With the aid of a second stitch, the skin forming the lower edge of the cut-out window is drawn tightly to the foundation bar (the needle with the thread is passed through the lower edge of the aperture under the introduced camera, and is then drawn above the lower border of the foundation bar (4) through the intact skin, after which the needle is introduced from the outside into the skin at the same border of the foundation bar and is drawn out by the side where it was first introduced; the free ends of the thread are tied into a knot and so the skin on the side of the aperture is found drawn to the skin on the opposite side). Following this, the skin at the edges of the aperture is trimmed to correspond to the camera dimensions; to the foundation bar, with the aid of two bolts having openings at the bar edges (5), the second additional foundation bar is mounted, and thus the skin fold with the camera becomes fixed in a vertical position. As the fascia on the side of the intact skin have been removed, the camera comes into direct contact with the blood vessels.

It can be seen that in our proposed method we are offering not a camera occupying a certain volume of space, but a transparent glass sheet, fixed into an aperture and replacing the removed skin with the aid of two bars vertically supporting the depilated skin. For this reason, the term "camera" should be accepted in our case as having a special connotation.

Microscopic observations are made without the aid of narcosis: the mouse with the attached camera (Fig. 2, I)

is immobilized with the aid of a special fixator as represented in Fig. 2, II. This fixator consists of a tube with a prolonged slit in its wall (II, B) and rings with forks fastened on it (II, A); the ring with the attached fork is placed on the tube in such a manner that the lower edge of the fork fits into the slit of the tube. The mouse is placed within the lumen of the fixator and the camera is placed vertically and moved along the slit of the tube, while, in order to immobilize the fork totally as it lies on the fixator, we have placed it between the head of the bolt and the bar; at the end opposite the camera, where it protrudes from the slit of the fixator, the rubber ring lying over the fixator is snugly fitted (Fig. 2, III). Microscopic observations are most conveniently made when the object table has been removed from the instrument; the camera in that case lies directly on the condenser.

We prepared 80 mice in the manner just described, and continued skin observations for 30 days and more. The camera permits picture-taking at various magnifications. In the small and large vessels the blood circulation was visible; in the capillaries and the arteries the walls were seen clearly; it is more difficult to demonstrate venous vessel walls.

The method described can be used for the study of the blood supply and growth of skin grafts placed in the skin folds as, for example, malignant tumors. Skin transplants can be performed with the aid of a thick needle supplied with an obturator. A bit of the tissue to be transplanted is placed within the lumen of the needle and then the needle is introduced on the window side and the end is pushed between the skin and glass plate and then, about in the middle of the latter, the piece of tissue is pushed out with the obturator. Then, to the outer surface of the foundation bar, BF-2 glue is used to fasten a cover glass over the window on the bar so that, in this manner, the skin is contained between two transparent sheets. In such a case, we are dealing with a camera whose slit actually consists of the fold of mouse skin.

SUMMARY

A modified transparent camera for observation is described. The operation of placing the camera is technically simple and excludes any damage to blood circulation in the skin. Observations through the camera may last for 30 days and more. Microphotography with various magnifications might be accomplished through this camera. The blood stream and the vascular walls are clearly seen. The camera might also be used in studies of tissues transplanted into skin folds.

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